National Library
of Canada

Canadian Theses Service

Ottawa, Canada
K1A 0N4

Bibliothèque nationale
du Canada

Service des thèses canadiennes

NOTICE

The quality of this microform is heavily dependent upon the quality of the original thesis submitted for microfilming. Every effort has been made to ensure the highest quality of reproduction possible.

If pages are missing, contact the university which granted the degree.

Some pages may have indistinct print especially if the original pages were typed with a poor typewriter ribbon or if the university sent us an inferior photocopy.

Previously copyrighted materials (journal articles, published tests, etc.) are not filmed.

Reproduction in full or in part of this microform is governed by the Canadian Copyright Act, R.S.C. 1970, c. C-30.

AVIS

La qualité de cette microforme dépend grandement de la qualité de la thèse soumise au microfilmage. Nous avons tout fait pour assurer une qualité supérieure de reproduction.

S'il manque des pages, veuillez communiquer avec l'université qui a conféré le grade.

La qualité d'impression de certaines pages peut laisser à désirer, surtout si les pages originales ont été dactylographiées à l'aide d'un ruban usé ou si l'université nous a fait parvenir une photocopie de qualité inférieure.

Les documents qui font déjà l'objet d'un droit d'auteur (articles de revue, tests publiés, etc.) ne sont pas microfilmés.

La reproduction, même partielle, de cette microforme est soumise à la Loi canadienne sur le droit d'auteur, SRC 1970, c. C-30.

NL-339 (r. 88/04)

Canada

THE UNIVERSITY OF ALBERTA

The behaviour and mode of action of chlorsulfuron in a susceptible species, *Thlaspi arvense* L.

by

Hendrik D. Bestman

A THESIS
SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH
IN PARTIAL FULFULMENT OF THE REQUIREMENTS FOR THE DEGREE OF
Doctor of Philosophy
IN
WEED SCIENCE

DEPARTMENT OF PLANT SCIENCE

EDMONTON, ALBERTA

Fall, 1988

Permission has been granted to the National Library of Canada to microfilm this thesis and to lend or sell copies of the film.

The author (copyright owner) has reserved other publication rights, and neither the thesis nor extensive extracts from it may be printed or otherwise reproduced without his/her written permission.

L'autorisation a été accordée à la Bibliothèque nationale du Canada de microfilmer cette thèse et de prêter ou de vendre des exemplaires du film.

L'auteur (titulaire du droit d'auteur) se réserve les autres droits de publication; ni la thèse ni de longs extraits de celle-ci ne doivent être imprimés ou autrement reproduits sans son autorisation écrite.

ISBN 0-315-45448-2

THE UNIVERSITY OF ALBERTA

RELEASE FORM

NAME OF AUTHOR Hendrik D. Bestman

TITLE OF THESIS **The behaviour and mode of action of chlorsulfuron in a susceptible species, *Thlaspi arvense* L.**

DEGREE FOR WHICH THESIS WAS PRESENTED Doctor of Philosophy

YEAR THIS DEGREE WAS GRANTED Fall 1988

Permission is hereby granted to THE UNIVERSITY OF ALBERTA to reproduce single copies of this thesis and to lend or sell such copies for private, scholarly or scientific research purposes only.

The author reserves other publication rights, and neither the thesis nor extensive extracts from it may be printed or otherwise reproduced without the author's written permission.

(signed) Henk Bestman

PERMANENT ADDRESS:

10319 - 155 Street
Edmonton, Alberta
T5P 2M1

Dated Oct 14, 1988

THE UNIVERSITY OF ALBERTA

FACULTY OF GRADUATE STUDIES AND RESEARCH

The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research, for acceptance, a thesis entitled **The behaviour and mode of action of chlorsulfuron in a susceptible species, *Thlaspi arvense* L.** submitted by Hendrik D. Bestman in partial fulfilment of the requirements of the degree of Doctor of Philosophy in Weed Science.

Supervisor

Arnost Horak

Edwin A. Cossins.

John Hoddinott

Stephen O. Duke

External examiner

Date 14 Oct, 1988

ABSTRACT

Stinkweed is susceptible to the herbicide chlorsulfuron. Seedlings slowly metabolized the herbicide to nonphytotoxic products. At 24 and 120 h after application, 88 and 45%, respectively, of the applied chlorsulfuron was still in the phytotoxic form. Chlorsulfuron reduced shoot growth of stinkweed. A spot application of 7 ng (20 picomoles) per seedling to the foliage was sufficient to result in a 50% reduction in plant height fourteen days later. Foliar absorption of chlorsulfuron by stinkweed seedlings was slow; 12 - 25% of the applied amount in 24 h. Translocation of the herbicide out of the treated leaf was limited; 1.7 - 2.8% of the applied amount in 24 h. One of the effects of chlorsulfuron treatment was a reduction in the incorporation of ^{14}C into amino acids in shoot apical tissue, when the shoot was exposed to $^{14}CO_2$. In both intact plants and excised leaves, chlorsulfuron reduced the export of assimilates out of herbicide-treated leaves. Twelve hours after a spot application of 1 μg, assimilate translocation was reduced by 30%. In chlorsulfuron-treated excised leaves, the concentration of sucrose had increased from 1.7 to 4.2 nmoles/mg, and the concentration of amino acids from 16.8 to 28.6 nmoles/mg, 30 h after application of the herbicide. Incorporation of ^{14}C-activity into free amino acids and into amino acids derived from soluble proteins extracted from chlorsulfuron-treated leaves, exposed to $^{14}CO_2$ 24 h after application of the herbicide, was reduced 36 and 43%, respectively. Supplying branched-chain amino acids to stinkweed seedlings prior to the application of chlorsulfuron prevented the occurrence of all effects described. The observed reduction in assimilate transport is not due to an effect on the synthesis of assimilates, but on their movement out of the herbicide-treated leaves. It is suggested that the limited phloem mobility of chlorsulfuron is due to the effect of the herbicide on the export of assimilates.

ACKNOWLEDGEMENTS

I want to express my sincere thanks to Dr. W.H. Vanden Born for enabling me to pursue graduate studies towards a Ph. D. degree under his supervision. His willingness to procure the necessary funds and to give me the freedom to investigate matters of my own choosing are much appreciated. His questions and editorial suggestions have contributed significantly to this thesis.

I owe much to Dr. M.D. Devine. In many ways this thesis is one of the products of our collaboration during the past six years. Our mutual desire to understand the mode of action of herbicides on a physiological and biochemical level has been the basis for countless stimulating discussions.

I thank Mary Packer, Ian Duncan, and Barry Zytaruk for the technical advice and assistance they have provided, and for their willingness to lend me supplies and equipment. The assistance of Ray Schraa in collecting the seed, in ordering supplies, and with the photography, is much appreciated. Chris Bladen is thanked for doing a lot of the tedious work associated with this research.

The University of Alberta is thanked for the use of their equipment and facilities, and, together with the Natural Science and Engineering Research Council of Canada and Alberta Agriculture (Farming for the Future), for the financial assistance.

E. I. Du Pont de Nemours & Co. Inc. supplied the formulated, technical, and radiolabelled chlorsulfuron.

My colleagues at The King's College are thanked for their words of encouragement during the last year.

This thesis is dedicated to Miranda and to our precious children, Reuben and Andrea. Only they know and have experienced how much this thesis has been a part of me.

TABLE OF CONTENTS

Chapter Page

1. INTRODUCTION 1

2. LITERATURE REVIEW 3
  2.1 The mode of action of herbicides 3
  2.2 Chlorsulfuron 4
    2.2.1 Agronomic use 5
    2.2.2 Chemistry, toxicity, and structure-activity relationship 9
    2.2.3 Absorption and translocation 12
    2.2.4 Mode of action 13
    2.2.5 Resistance 17
    2.2.6 Effects on plant metabolism 19
    2.2.7 Selectivity 20
  2.3 Other amino acid biosynthesis-inhibiting herbicides 21
    2.3.1 Imidazolinones 22
    2.3.2 Glyphosate 24
    2.3.3 Aminotriazole 27
    2.3.4 Phosphinothricin 27

3. MATERIALS AND METHODS 28
  3.1 General 28
    3.1.1 Plant material 28
    3.1.2 Nutrient solutions 28
    3.1.3 Herbicide 28
    3.1.4 Assay procedures 30

3.1.4.1 Sugar quantification 30
3.1.4.2 Amino acid quantification 30
3.1.4.3 Sample combustion and liquid scintillation spectrometry 31
3.2 Dose response 32
3.3 Growth measurements 32
3.3.1 Increase in shoot fresh weight 32
3.3.2 Rate of leaf elongation 33
3.4 Translocation of chlorsulfuron 35
3.4.1 ^{14}C-Chlorsulfuron translocation 35
3.4.1.1 Foliage application 35
3.4.1.2 Root application 36
3.4.2 Serial mutilation 36
3.5 Metabolism of chlorsulfuron 37
3.5.1 Method 37
3.5.1.1 Extraction procedure 37
3.5.1.2 Chromatography 39
3.5.1.3 Beta-glucosidase assay 40
3.5.1.4 Herbicidal activity assay 41
3.5.2 Extent of chlorsulfuron metabolism in stinkweed seedlings 41
3.6 CO_2-fixation and assimilate translocation 42
3.6.1 Design and construction of assimilation chamber 42
3.6.2 Experimental procedure 42
3.6.3 $^{14}CO_2$-labelling procedure 43
3.7 Intact seedling experiments 45
3.7.1 Incorporation of ^{14}C-activity into various fraction following exposure to $^{14}CO_2$ 45
3.7.2 Assimilate translocation 46
3.7.2.1 Chlorsulfuron and $^{14}CO_2$ applied to the same leaf 46

3.7.2.2 Chlorsulfuron and $^{14}CO_2$ applied to adjacent leaves 46

3.7.2.3 Uptake of ^{14}C-leucine by the roots and distribution of ^{14}C-activity 46

3.8 Exudation from excised leaves 47

3.8.1 Exudation profiles following chlorsulfuron treatment and exposure to $^{14}CO_2$ 48

3.8.1.1 Chlorsulfuron and $^{14}CO_2$ applied to the same leaf 48

3.8.1.2 Chlorsulfuron and $^{14}CO_2$ applied to separate leaves 48

3.8.3 Exudation profiles following chlorsulfuron treatment and application of ^{3}H-sucrose and ^{14}C-sucrose 49

3.8.4 Incorporation of ^{14}C-activity into various fractions following exposure to $^{14}CO_2$ 50

3.8.5 Incorporation of ^{14}C-activity into soluble leaf proteins following exposure to $^{14}CO_2$ 50

3.9 Extraction, separation, and analysis of various fractions from plant tissues 50

3.9.1 Extraction procedure 50

3.9.2 Fractionation of water-alcohol fraction 51

3.9.3 Analysis of sugar fraction 52

3.9.4 Analysis of amino acid fraction 52

3.9.5 Fractionation of lipid fraction 52

3.9.6 Fractionation of pellet 52

3.10 Protein extraction, quantification, and separation 53

3.10.1 Extraction procedure 53

3.10.2 Protein quantification and specific activity determination 53

3.10.3 Polyacrylamide gel electrophoresis 54

3.10.4 Fluorography 55

3.10.5 Protein hydrolysis 55

4. RESULTS AND DISCUSSION 56

4.1 Response of stinkweed seedlings to increasing doses of chlorsulfuron 56

4.2 Effect of chlorsulfuron on the growth of stinkweed seedlings........................56

4.2.1 Increase in shoot fresh weight........................58

4.2.2 Rate of elongation of the fifth true leaf........................59

4.2.3 Discussion........................62

4.3 Absorption and translocation of chlorsulfuron in stinkweed seedlings........................63

4.3.1 Foliar application........................63

4.3.2 Root application........................66

4.3.3 Comparison between foliar and root application........................66

4.3.3 Mutilation study........................70

4.3.4 Discussion........................71

4.4 Metabolism of chlorsulfuron........................73

4.4.1 Procedure........................74

4.4.1.1 Extraction........................74

4.4.1.2 Chromatography........................75

4.4.1.3 Identification of the fractions........................78

4.4.2 Extent of chlorsulfuron metabolism in stinkweed seedlings........................80

4.4.3 Discussion........................81

4.5 Effect of chlorsulfuron on the allocation of ^{14}C-activity in intact seedlings following exposure to $^{14}CO_2$........................83

4.5.1 Recovery and allocation of ^{14}C-activity in fractions extracted from nontreated shoot tissue........................83

4.5.2 Translocation of ^{14}C-activity into chlorsulfuron-treated leaves........................86

4.5.3 Discussion........................86

4.6 Effect of chlorsulfuron on assimilate translocation........................87

4.6.1 Intact seedlings........................87

4.6.2 Excised leaves........................88

4.6.2.1 Optimum EDTA concentration........................89

4.6.2.2 Exudation following $^{14}CO_2$-application........................90

4.6.2.3 Exudation following ^{3}H-sucrose and ^{14}C-sucrose application 95

4.6.3 Discussion 98

4.7 Effect on incorporation of ^{14}C-activity into various fractions following exposure to $^{14}CO_2$ 99

4.8 Effect on incorporation of ^{14}C-activity into soluble proteins following exposure of excised leaves to $^{14}CO_2$ 103

4.9 General discussion 106

5. SUMMARY AND CONCLUSIONS 112

BIBLIOGRAPHY 115

APPENDIX 122

LIST OF TABLES

Table Page

1. Sulfonylurea herbicides....................6
2. Nomenclature and physicochemical properties of chlorsulfuron....................10
3. Herbicides that inhibit amino acid biosynthesis....................22
4. Absorption and translocation of chlorsulfuron in stinkweed following application to the foliage or the roots....................68
5. Accumulation of chlorsulfuron in stinkweed seedlings following application to the foliage or to the roots....................69
6. The effect of removal of the treated leaf of stinkweed seedlings at different times after an application of 1 µg chlorsulfuron, on the increase in shoot dry weight, assessed 14 days after herbicide treatment....................71
7. Distribution of ^{14}C-activity recovered from flax, stinkweed, and wheat treated with ^{14}C-chlorsulfuron....................75
8. Distribution of ^{14}C-activity among methanol fractions of the extracts of flax, stinkweed, and wheat treated with ^{14}C-chlorsulfuron....................77
9. The effect of methanol fractions of extracts of chlorsulfuron-treated flax, stinkweed, and wheat extracts on the growth of stinkweed seedlings....................80
10. Metabolism of chlorsulfuron in stinkweed seedlings, assessed 12 and 24 h after the application of ^{14}C-chlorsulfuron to a single leaf....................82
11. Effect of chlorsulfuron on recovery and allocation of ^{14}C-activity in the various fractions extracted from non-treated shoots following exposure to $^{14}CO_2$....................84
12. Effect of chlorsulfuron on recovery and allocation of ^{14}C-activity in the various fractions extracted from shoot apical tissue following exposure of the shoot to $^{14}CO_2$....................85
13. Effect of chlorsulfuron on assimilation and exudation by excised leaves of stinkweed seedlings, and the allocation of ^{14}C-activity in various fractions extracted from these leaves following exposure to $^{14}CO_2$....................100
14. Effect of chlorsulfuron on the amounts of sucrose, glucose, and fructose and their specific activities following exposure of excised stinkweed leaves to $^{14}CO_2$....................102
15. Effect of chlorsulfuron on the amount of soluble proteins and on the incorporation of ^{14}C-activity into these proteins following exposure of excised leaves of stinkweed seedlings to $^{14}CO_2$....................104
16. Comparison between the effect of chlorsulfuron on assimilate translocation in intact seedlings, on the exudation by excised leaves, and on the concentration of chlorsulfuron in the assimilate source tissue....................110

LIST OF FIGURES

Figure Page

1. Basic chemical structure of sulfonylurea herbicides 12
2. Metabolic pathways of chlorsulfuron in wheat and flax 21
3. Assembly of flask and balance used to weigh the shoots of stinkweed seedlings 33
4. Radial transducer monitoring the rate of leaf elongation of a stinkweed seedling 34
5. Flow diagram of extraction, separation, and quantification of ^{14}C-chlorsulfuron and its metabolites from plant tissue 38
6. Assembled assimilation chamber consisting of inner and outer compartment 44
7. Baseplate of assimilation chamber with six glass cups for nutrient solution, the syringes connected with Teflon™ lines to each cup, and the Teflon™ air lines for the inner and outer compartment 44
8. Stinkweed seedlings mounted in assimilation chamber 45
9. Effect of chlorsulfuron on the height, fresh weight, and dry weight of stinkweed seedlings, 14 days after application to the third true leaf 57
10. Effect of 1 μg of chlorsulfuron, applied to the third true leaf of stinkweed seedlings, on the increase in shoot fresh weight 58
11. Elongation of the fifth true leaf of stinkweed seedlings growing in nutrient solution containing 1 μg/ml chlorsulfuron, and with or without 1 mM of each of the amino acids L-valine, L-leucine, and L-isoleucine 60
12. Elongation of the fifth true leaf of stinkweed seedlings growing in nutrient solution containing 1 mM of each of the amino acids L-valine, L-leucine, and L-isoleucine 61
13. Elongation of the fifth true leaf of stinkweed seedlings growing in nutrient solution 61
14. Absorption and translocation of ^{14}C-chlorsulfuron in stinkweed seedlings 64
15. Distribution of ^{14}C-activity in stinkweed seedlings following appliction of ^{14}C-chlorsulfuron to either the third or fourth true leaf 65
16. Accumulation of chlorsulfuron in stinkweed seedlings via uptake from nutrient solution containing 1 μg/ml chlorsulfuron 67

17. Representative chromatograms obtained by reverse-phase separation of ^{14}C-chlorsulfuron and extracts of ^{14}C-chlorsulfuron-treated flax, stinkweed, and wheat, using a step gradient of aqueous 0.1% (v/v) formic acid and methanol....76

18. Effect of 1 μg of chlorsulfuron, applied to a single leaf of intact stinkweed seedlings, on the translocation of ^{14}C-assimilates out of the treated leaf, of the leaf adjacent to the treated leaf....88

19. Effect of increasing concentration of EDTA in the bathing solution on the exudation of anthrone-positive compounds by the petioles of excised leaves of stinkweed seedlings....89

20. Exudation profiles of single, excised stinkweed leaves, following application of blank or 1 μg of chlorsulfuron to the leaves, 6, 12, or 24 h prior to excision and exposure to $^{14}CO_2$ 91

21. Exudation profiles of single, excised stinkweed leaves, following application of blank or 1 μg of chlorsulfuron to the leaves 24 h prior to excision and exposure to $^{14}CO_2$ 92

22. Exudation profiles of single, excised stinkweed leaves, following a spray application of 1 or 11.25 g/ha of chlorsulfuron, 24 h prior to excision and exposure to $^{14}CO_2$ 93

23. Exudation profiles of single, excised stinkweed leaves, following application, 24 h prior to excision and exposure to $^{14}CO_2$, of blank or 1 μg of chlorsulfuron to the leaf adjacent to the excised leaf....94

24. Exudation profiles of single, excised stinkweed leaves, following application of 0 or 1 μg of chlorsulfuron to the leaves, 12 h prior to the treatment with ^{3}H- and ^{14}C-sucrose. 96

25 Exudation profiles of single, excised stinkweed leaves, following application of 0 or 1 μg of chlorsulfuron to the leaves, 24 h prior to the treatment with ^{3}H- and ^{14}C-sucrose. 97

26 Combined SDS-PAGE and corresponding fluorogram of soluble proteins extracted from excised leaves of stinkweed seedlings exposed to $^{14}CO_2$ 105

27 Diagram illustrating the relationship between the mode of action of chlorsulfuron, the fixation of carbon dioxide, and the translocation of assimilate from source to sink tissue. 107

1. INTRODUCTION

During the last few decades, chemical weed control has become an increasingly important crop management practice for many farmers. The use of herbicides on the Canadian prairie provinces alone has increased from 2,238,000 kg in 1958 to 14,233,000 kg in 1978 (Hay, 1980). Understanding the behaviour and action of these chemicals is basic to any recommendation for their safe use. Mode of action[1] studies can provide information regarding the time and manner of application, species susceptibility, and the manner in which susceptible plants die.

In addition, the study of the mode of action of herbicides has contributed greatly to the understanding of plant metabolism. For example, the current knowledge of plant photosynthesis has been gained in large measure through the availability of specific inhibitors of electron transport. The herbicides diuron and atrazine are such inhibitors. Many other herbicides are or have the potential to become important tools for studying the physiology and biochemistry of plants. The recently registered herbicide chlorsulfuron has the potential to become such a tool.

The overall objective of this study was to develop an understanding of the mode of action of the herbicide chlorsulfuron in a susceptible annual broadleaved weed [stinkweed (*Thlaspi arvense* L.)]. To attain this objective, attention was focused primarily on the events that occur during the first 24 h after foliage application of the herbicide. Several key questions have guided this study: (a) What is the effect of chlorsulfuron on the growth of stinkweed seedlings? (b) Is chlorsulfuron absorbed by stinkweed and is it subsequently translocated within the seedling? (c) Does stinkweed metabolize chlorsulfuron to non-phytotoxic compounds? (d) Does chlorsulfuron affect the metabolism of carbon compounds in stinkweed? (e) Is assimilate transport affected by chlorsulfuron?

---

[1] Throughout this study the term "mode of action" is used to denote the whole complex of herbicide-plant interaction; "mechanism of action" indicates a specific metabolic pathway or process that is affected directly by the herbicide; "site of action" is reserved for the specific site at which the herbicide molecules act.

The selection of stinkweed as the main experimental species was based on its convenient size for experimental purposes, its high susceptibility to chlorsulfuron, and the availability of sufficient seed to accomplish this study. Flax (*Linum usitatissum*) and wheat (*Triticum aestivum*) were included in the metabolism studies because of their ability to metabolize chlorsulfuron to non-phytotoxic products.

2. LITERATURE REVIEW

2.1 The mode of action of herbicides

The herbicidal action of a chemical arises from its ability to interact with a plant in such a manner as to inhibit or disturb its growth in a detrimental manner. This interaction usually involves an inhibition or disturbance of, or effect on, a process essential to the growth of that plant. Based on what is known about the mode of action of herbicides, several such interactions can be identified. For example, the herbicidal effects of some chemicals can be attributed to their effect on photosynthesis. For others the effect is on carotenoid biosynthesis. The effect of some herbicides can be attributed to their ability to uncouple the synthesis of ATP from electron transport. A few herbicides are known to interfere directly with protein or nucleic acid biosynthesis. Others interfere with microtubule formation or function during cell division. Still others affect auxin metabolism.

According to Fedtke (1982), in the study of the mode of action of herbicides, six different types of data can be gathered, each yielding its own unique information about the interaction between herbicide and plant. Data about the time and method of application required to obtain a herbicidal effect provide information about the sensitive growth stage and sensitive part of the plant. A survey of the sensitive tissue yields information regarding the cell type affected by the herbicide. Cytological and microscopic observations might provide clues as to which cellular and subcellular components are affected. In addition, by using autoradiographic or microautoradiographic techniques, information about possible sites of herbicide accumulation in the plant can be gained. Investigations at the physiological level can demonstrate the effect of the herbicide on *in vivo* plant metabolic processes. Biochemical experiments, such as *in vitro* investigations with isolated enzyme systems, provide data on the influence of the herbicide on a particular metabolic pathway. Biochemical parameters such as the concentration of plant metabolites can also provide the investigator with clues regarding the effect of the herbicide on metabolic pathways. Information about the possible interaction of the herbicide with a protein, either an enzyme or a binding protein, can provide evidence for the site of molecular interaction, i.e., the site of action.

In addition to the fact that the understanding of the mode of action of a herbicide is limited by a general lack of complete understanding of the physiological and biochemical processes involved in the growth of a plant, there is the difficulty of distinguishing between the primary effect of the herbicide at the site of action and its metabolic consequences, and the resulting secondary effects. Consequently, for many of the currently available herbicides the understanding of their mode of action is limited to a general knowledge of the time and method of application, the sensitive tissue, the distribution and accumulation of the herbicide within the whole plant following application, and a broad description of their influence on *in vivo* metabolic systems. In the case of some herbicides, detailed cytological work has been done. For others, a more detailed description of their effect on a metabolic pathway is available. In the case of only a few herbicides is there some understanding of their action at a molecular level. Chlorsulfuron is one of these herbicides.

2.2 Chlorsulfuron

Chlorsulfuron belongs to the sulfonylurea class of herbicides (Table 1; Blair and Martin, 1988). The herbicides in this recently developed group of compounds are noted for their low rates of application and their low mammalian toxicity. As a group they already have proved to be very interesting from the point of view of their structure-activity relationships. Slight modifications of their chemical structures have resulted in large differences in their selectivity patterns and their persistence in the soil. Chlorsulfuron and metsulfuron are both selective herbicides that are used for broadleaved weed control in cereal crops. Sulfometuron methyl is considerably less selective and is more persistent in soil. It is recommended primarily for long-term vegetation control.

2.2.1 Agronomic use

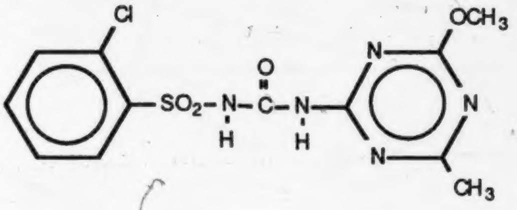
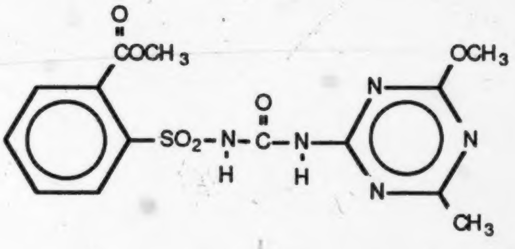
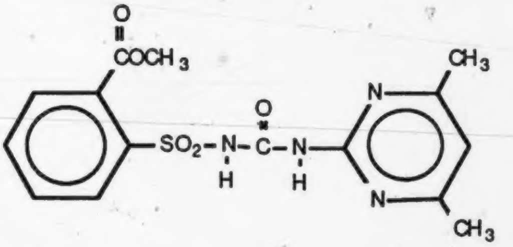
In Canada, chlorsulfuron is registered[2] for use in wheat, barley (*Hordeum vulgare*), oats (*Avena sativa*), and in reduced-tillage fallow, in the prairie provinces and in the Peace River region of British Columbia. Application of a postemergence spray of chlorsulfuron at a rate of 11 g/ha[3] controls such broadleaved weeds as lamb's quarters (*Chenopodium album* L.), hempnettle (*Galeopsis tetrahit* L.), wild mustard [*Brassica kaber* (DC.) L. C. Wheeler], volunteer rapeseed (*Brassica campestris* L.), flixweed [*Descurania sophia* (L.) Webb], redroot pigweed (*Amaranthus retroflexus* L.), stinkweed, green smartweed (*Polygonum scabrum* Moench), lady's thumb (*Polygonum persicaria* L.) and cow cockle (*Saponaria vaccaria* L.). At the higher recommended rate, 22.5 g/ha, chlorsulfuron also controls chickweed [*Stellaria media* (L.) Vill.], kochia [*Kochia scoparia* (L.) Schrader], cleavers (*Galium aparine* L.), Canada thistle [*Cirsium arvense* (L.) Scop.], wild buckwheat (*Polygonum convolvulus* L.), and Russian thistle [*Salsoli kali* (L.) var. *tenuifolia* Tausch.]. The addition of a surfactant to the spray solution is recommended.

---

[2] Registered under the trade name GLEAN. Registration no 17245, Pest Control Products Act. Canadian Patent no 1082189.

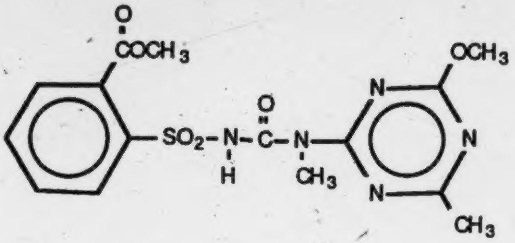
[3] Unless otherwise noted, herbicide application rates are recorded in grams of active ingredient per hectare.

Table 1. **Sulfonylurea herbicides**

Common name TRADE NAME (Code name)	Chemical name and structural formula
Chlorsulfuron GLEAN (DPX-W4189)	2-chloro-*N*-[(4-methoxy-6-methyl-1,3,5-triazin-2yl)amino-carbonyl]benzenesulfonamide  Cl O SO$_2$–N–C–N H H N N N OCH$_3$ CH$_3$
Metsulfuron ALLY (DPX-T6376)	methyl 2[[[[(4-methoxy-6-methyl-1,3,5-triazin-2-yl) amino]carbonyl]amino]sulfonyl]benzoate  O COCH$_3$ O SO$_2$–N–C–N H H N N N OCH$_3$ CH$_3$
Sulfometuron methyl OUST (DPX-T5648)	Methyl 2-[[[[(4,6-dimethyl-2-pyrimidinyl)amino]-carbonyl]amino]sulfonyl]benzoate  O COCH$_3$ O SO$_2$–N–C–N H H N N CH$_3$ CH$_3$

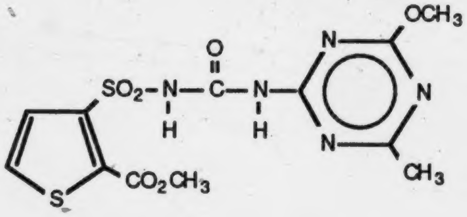
EXPRESS
(DPX-L5300)

2-[3-(4-methoxy-6-methyl-1,3,5-triazin-2yl)-3-methylureidosulfonyl]benzoate

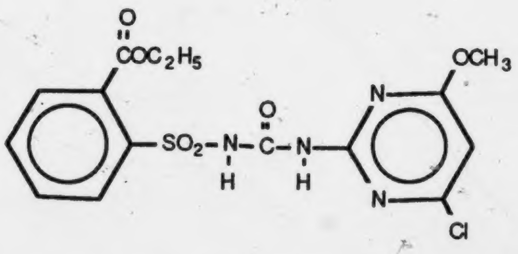


REFINE
(DPX-M6316)

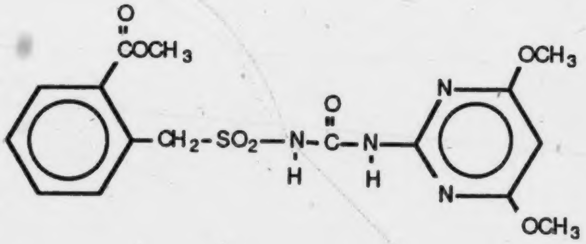
Methyl 3-[[(4-methoxy-6-methyl-1,3,5-triazin-2-yl) aminocarbonyl]aminosulfonyl]-2-thiophenecarboxylate



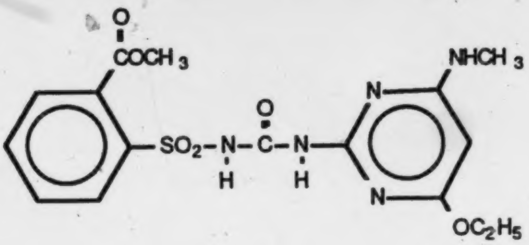
Chlorimuron ethyl
CLASSIC
(DPX-F6025)



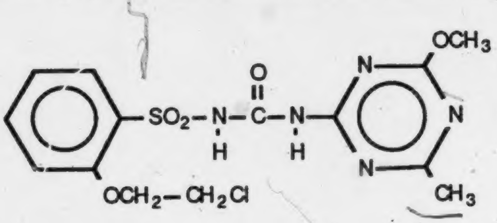
Bensulfuron methyl
LONDAX
(DPX-F5384)



MUSTER (DPX-A7881)	2-[[[[4-ethoxy-6-(methylamino)-1,3,5-triazin-2-yl]amino]-carbonyl]amino]sulfonyl]benzoate



Triasulfuron AMBER (CGA 131-036)	*N*-(6-methoxy-4-methyl-1,3,5-triazin-2-yl-amino-carbonyl)-2-(2-chloroethoxy)-benzenesulfonamide



Chlorsulfuron can be mixed with difenzoquat (1,2-dimethyl-3,5-diphenyl-1*H*-pyrazolium methyl sulfate), diclofop methyl (methyl 2-[4-(2,4-dichlorophenoxy)-phenoxy]-propanoate), or flamprop methyl (methyl(±)-2-[*N*-(3-chloro-4-fluorophenyl)benzamido]-propionate), enabling a farmer to control chlorsulfuron-susceptible broadleaved weeds and wild oats (*Avena fatua* L.) in cereal crops with a single spray application.

Chlorsulfuron is recommended only for use on cropland that is dedicated primarily to the production of wheat, barley, and oats, and that has a soil pH of 7.5 or less. The soil residual activity of chlorsulfuron can injure such crops as rapeseed, lentils (*Lentilla lens*), field beans (*Phaseolus vulgaris*), peas (*Pisum sativum*), flax, sugarbeet (*Beta vulgaris*), and potatoes (*Solanum tuberosum* L.). The minimum

recropping interval[4] on soils with a pH of 7.0 or less is 2 months for spring and winter wheat, and 10 months for durum wheat, barley, and oats. On soils with a pH of 7.1 to 7.5 this interval is 2 months for spring and winter wheat, 10 months for durum wheat, and 22 months for barley and oats.

2.2.2 Chemistry, toxicity, and structure-activity relationship

Chlorsulfuron can be synthesized readily by reacting equivalent amounts of 2-chlorobenzenesulfonyl isocyanate with 2-amino-4-methoxy-6-methyl-1,3,5-triazine in acetonitrile at room temperature (Levitt *et al.*, 1981). The resulting mixture is filtered and the precipitate is washed with ethyl ether to yield the desired product. The physico-chemical properties of chlorsulfuron are presented in Table 2 (Anonymous, 1983).

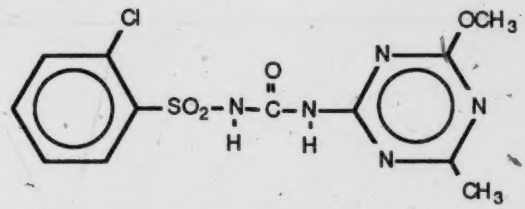
Chlorsulfuron is a weak acid that has been observed to degrade by hydrolysis. This hydrolysis is pH-dependent and occurs fastest at pH levels below 7. The hydrolysis products are 2-chlorobenzene sulfonamide and 2-amino-4-methoxy-6-methyl-1,3,5-triazine. Herrmann *et al.* (1985) reported half-lives for chlorsulfuron, under indoor conditions, of 92 h in methanol, 78 h in distilled water, and 18 h in natural creek water. The photostability of chlorsulfuron was studied by exposing chlorsulfuron adsorbed to silica gel or montmorillonite powder to irradiation (< 290 nm) for 6 h. The half-lives were 60 and 66 h, respectively. Under outdoor conditions, half-lives for chlorsulfuron were 186 h in distilled water, 31 h in creek water, 136 h when adsorbed to silica gel, and 115 h when adsorbed to montmorillonite powder (Herrmann *et al.*, 1985).

---

[4] The minimum recropping interval is the time from last chlorsulfuron application to the anticipated date of planting.

Table 2. Nomenclature and physicochemical properties of chlorsulfuron

Chemical name:	2-chloro-N-[(4-methoxy-6-methyl-1,3,5-triazin-2-yl)amino carbonyl]benzenesulfonamide
Common name:	chlorsulfuron
Product name:	GLEAN Weed Killer 75% Dry Flowable
Manufacturer:	DuPont
Structural formula:	



Molecular formula:	$C_{12}H_{12}ClN_5O_4S$
Molecular weight:	357.8
Physical description:	odourless, white, crystalline solid
Melting point:	174-178 C
Decomposition temperature:	192 C
Vapour pressure:	4.6×10^{-6} mm Hg at 25 C
pK_a:	4.6

1-Octanol/water partition coefficient:

pH	Log P
4.5	1.05
12.0	-0.67

Solubility:

Solvent	Temp. C	g/100 ml
Acetone	22	5.7
Hexane	22	-
Methanol	22	1.4
Methylene chloride	22	10.2
Toluene	22	0.3
Water (pH 5)	25	0.03
Water (pH 7)	25	2.8

Chlorsulfuron is of comparatively low mammalian toxicity (Anonymous, 1979). Its oral LD_{50} for fasted male rats is 5545 mg/kg. The herbicide has a low toxicity to wildlife and fish [oral LD_{50} >5000 mg/kg for quail and duck, LC_{50} (96 h) >250 ppm for trout and blue gill], it has shown no mutagenic activity in the Ames test, and its rates of excretion from mammals are very high (99.9% within 3 days) (Herrmann *et al.*, 1985). Neither chlorsulfuron nor its hydrolysis or metabolic products have shown any potential accumulation through the food chain (Anonymous, 1980).

The sulfonylurea compounds can be viewed as a combination of three moieties; aryl ring, sulfonylurea bridge, and heterocyclic ring (Figure 1). In the development of chlorsulfuron, optimization of the structure for herbicidal activity was accomplished by varying one of these moieties at a time (Levitt, 1982). In the aryl ring, ortho substitution (# 2) resulted in the highest activity, followed by substitution at the meta position (# 3). Substitution at the para position (# 4) produced inactive compounds. The substituent can be a variety of atoms or small groups, except for a free carboxylic group. Disubstitution at the two ortho positions (# 2 and 6) with chlorine (Cl) produced a compound with the same activity as one that had a single Cl substitution at one of the ortho positions.

With respect to the heterocyclic moiety of the molecule, the highest activity was obtained with compounds that had a methoxy ($-OCH_3$) group in the X position and a methyl ($-CH_3$) group in the Y position. Substitution at the 5-position resulted in lower herbicidal activity. The bridge connection at the # 2 position resulted in compounds that had the highest activity. The greatest activity was obtained when Z was a nitrogen (N) atom. Compounds with unmodified sulfonylurea bridges had the highest activity.

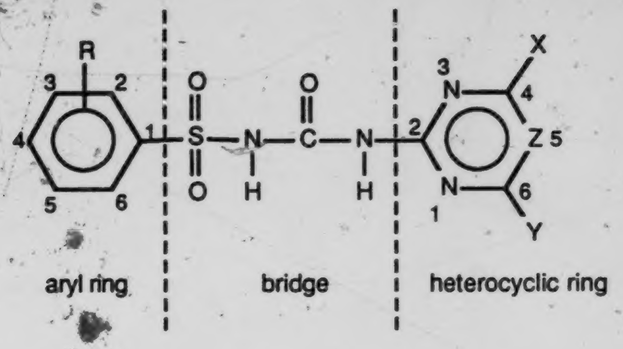


Figure 1. Basic chemical structure of sulfonylurea herbicides

2.2.3 Absorption and translocation

A wide range of values are reported for the absorption of chlorsulfuron by the foliage of plants. Sweetser *et al.* (1982) reported that 24 h after the application of ^{14}C-chlorsulfuron, on average 84% of the applied ^{14}C-activity was absorbed by leaves of susceptible sugarbeet, soybean, mustard, and cotton. In tolerant wheat, barley, and wild oats, 65% of the applied activity was absorbed during the same time period. Hageman and Behrens (1984a) found less absorption by eastern black nightshade (*Solanum ptycanthum* Dun.) and velvetleaf (*Abutilon theophrasti* Medic.). In these two species, on average approximately 40% of the recovered ^{14}C-activity had been absorbed by the leaves, 24 h after the application of ^{14}C-chlorsulfuron.

Devine *et al.* (1984) have demonstrated that the determination of the amount of herbicide absorbed by leaf tissue depends greatly on the method employed. Their work with several species, leaf wash solutions, and wash techniques, suggests that rinsing a treated leaf twice with, or dipping it twice in, 10% (v/v) aqueous ethanol, effectively removes all of the unabsorbed herbicide. Using the rinse or dip technique they found that, 24 h after the application of ^{14}C-chlorsulfuron, approximately 20% of the recovered activity was absorbed by leaves of Tartary buckwheat. In the case of barley and Canada thistle, the values were 37 and 13%, respectively.

Chlorsulfuron, or its metabolite(s), is translocated in both tolerant and susceptible species. Sweetser *et al.* (1982) observed no difference between tolerant species (wheat, barley, and wild oats) and susceptible species (sugarbeet, mustard, soybean, and cotton) in the amount of ^{14}C-activity translocated out a leaf that had been treated with ^{14}C-chlorsulfuron. In all instances, on average approximately 74% of the applied ^{14}C-activity had been translocated out of the treated leaf, 24 h after application. In eastern black nightshade and velvetleaf, no difference was observed in the amount of ^{14}C-activity translocated out of the treated leaf 4, 8, and 12 h after application of ^{14}C-chlorsulfuron (Hageman and Behrens, 1984a). However, 24 and 48 h after application, more ^{14}C-activity had translocated out of the treated leaf of the tolerant species eastern black nightshade (68 and 80% of the absorbed activity, respectively) than of the susceptible species velvetleaf (49 and 57%, respectively).

2.2.4 Mode of action

One of the most noticeable responses of susceptible plants to a foliage or root application of chlorsulfuron is a rapid inhibition of growth. In one of the first reports on the mode of action of chlorsulfuron, Ray (1980) reported that 48 h after placing the roots of corn seedlings in a solution of 2.8 nM chlorsulfuron, the increase in root fresh weight was reduced. During the same time period the increase in shoot fresh weight was reduced by chlorsulfuron concentrations of 28 nM or greater. The growth rate of a leaf of corn seedlings whose foliage had been treated with chlorsulfuron was reduced within 2 h after treatment. Eight hours after treatment, the growth rate was only 20% of the growth rate at treatment time. These observations suggest that chlorsulfuron acts very rapidly (Ray, 1982b).

The rapid inhibition of growth is confirmed by observing the effect of chlorsulfuron on cell division in susceptible plants. In broadbean (*Vicia faba*) the mitotic index[5] of root tips was reduced from 6.4 to 0.9 division figures when the roots had been exposed to 2.8 nM chlorsulfuron (Ray, 1980). No effect on the frequency distribution of the various mitotic stages in chlorsulfuron-treated root tips was observed. Chlorsulfuron inhibits the incorporation of ^{3}H-thymidine into treated corn root and pea root tips. Four hours after treatment with 28 nM chlorsulfuron a 50% inhibition of ^{3}H-thymidine incorporation into corn root tips was observed (Ray, 1980). Observation of the effects of chlorsulfuron on the cell cycle of pea root tip meristem indicates that chlorsulfuron inhibits the progression of cells from the G2 phase (pre-mitotic) to the M phase (mitosis), and from the G1 phase (pre-DNA synthesis) to the S phase (DNA synthesis) (Rost, 1984). Chlorsulfuron does not appear to exert an effect during the mitotic phase and the DNA synthesis phase. Instead, it appears to act during the transition phases.

The effect of chlorsulfuron on the growth rate of susceptible plants is not due to an effect on cell elongation or expansion processes. Chlorsulfuron at concentrations up to 28 nM did not affect the indole acetic acid-induced elongation of etiolated pea stems nor the cytokinin-induced cell expansion in cucumber (*Cucumis sativus*) cotyledons, nor did it affect the gibberellic acid-induced elongation of lettuce (*Lactuca sativa*) hypocotyls (Ray, 1980).

In order to localize the site of action of herbicides, enzymatically isolated, metabolically active, leaf cells and chloroplasts can be used (Ashton *et al.*, 1977). When such cells obtained from soybean (*Glycine max*) were incubated for 30, 60, and 120 min with 0.1 to 100 μM chlorsulfuron, no effect on the incorporation of $^{14}CO_2$ was observed (Hatzios and Howe, 1982). In the same study no effect on the incorporation of ^{14}C-leucine into protein was observed, except at the highest (100 μM) concentration and after a 120-min incubation period. RNA synthesis, measured by the incorporation of ^{14}C-uracil, was inhibited only at 100 µM chlorsulfuron with incubation periods of 60 and 120 min. The incorporation of ^{14}C-acetate into lipids was inhibited by 1 µM or greater chlorsulfuron concentrations and after 30-min or longer incubation periods. Based on these results, Hatzios and Howe (1982) concluded that lipid biosynthesis was the most sensitive and the first metabolic process inhibited by chlorsulfuron.

---

[5] The number of dividing cells per 100 cells.

In similar experiments with leaf cells isolated from beans (*Phaseolus vulgaris* L.), de Villiers *et al.* (1980) found that incubation with 500 µM chlorsulfuron for 120 min had reduced the incorporation of $^{14}CO_2$ by 91%. Under the same conditions the synthesis of RNA, protein, and lipids was inhibited by 67, 59, and 64%, respectively. Respiration was reduced by 26%. Since chlorsulfuron does not affect the light-induced ATPase activity of isolated chloroplasts, de Villiers suggested that it inhibits photosynthesis by inhibiting PS II-mediated oxygen evolution. However, Ray's work (1980) indicates that oxygen evolution of chloroplasts incubated with 280 µM chlorsulfuron is not affected. Since the inhibition of $^{14}CO_2$ incorporation, as reported by de Villiers *et al.*, (1980) occurred at high concentrations of chlorsulfuron, it is concluded that photosynthesis is probably not the primary site of action of chlorsulfuron. Most photosynthesis-inhibiting herbicides exhibit their effect at much lower concentrations, i.e. 1 µM.

Although chlorsulfuron reduced the incorporation of ^{3}H-thymidine into the DNA of corn root cells, no direct effect on DNA synthesis has been demonstrated. Using nuclei isolated from etiolated soybean hypocotyl tissue, Ray (1982a) demonstrated that chlorsulfuron at concentrations up to 30 µM had no effect on the incorporation of ^{3}H-thymidine triphosphate. Neither was the activity of the enzyme DNA polymerase, extracted from corn roots, affected by chlorsulfuron. Chlorsulfuron did not affect the synthesis of the nucleosides required for DNA synthesis, nor was the phosphorylation of thymidine inhibited by the herbicide.

A major breakthrough in the understanding of the mode of action of chlorsulfuron, and of its analog sulfometuron methyl, occurred when it was demonstrated, both with bacteria and with higher plants, that the growth inhibition caused by these herbicides could be alleviated by the addition of amino acids to the growing medium (Anderson and Hibberd, 1985; LaRossa and Schloss, 1984; Ray, 1984a; Scheel and Casida, 1985). A combination of the branched-chain amino acids L-valine and L-isoleucine, each at a concentration of 100 µM in the growing medium, protected excised pea roots from the growth-inhibiting effects of chlorsulfuron. Chlorsulfuron, at concentrations up to 100 times that needed to cause inhibition of growth, did not affect the growth of pea roots in the presence of these two amino acids.

This ability of the valine-isoleucine combination to alleviate the chlorsulfuron-induced growth inhibition has been demonstrated also in whole plants (Ray, 1984a). When peas were germinated in the presence of 28 nM chlorsulfuron, nearly complete growth inhibition occurred. However, when 100 µM valine and isoleucine were included in the growing medium along with the chlorsulfuron, normal growth occurred. These results suggest that the mode of action of chlorsulfuron involves the biosynthesis of valine and isoleucine.

In subsequent experiments, Ray (1984a) and LaRossa and Schloss (1984) found that the enzyme acetolactate synthase (ALS; EC 4.1.3.18), the first common enzyme in the biosynthetic pathways of valine and isoleucine, was strongly inhibited by chlorsulfuron and metsulfuron methyl. As little as 28 nM chlorsulfuron inhibits the activity of this enzyme. There is no difference in the inhibitory effect of chlorsulfuron on acetolactate synthase extracted from susceptible or tolerant species. In both instances the activity of the enzyme is reduced by chlorsulfuron. This strongly suggests that the selectivity of chlorsulfuron is not due to differences in the target enzyme.

Three isozymes of ALS have been found in the enteric bacteria *Salmonella typhimurium* (ALS I and II) and *Escherichia coli* (ALS I and III) (De Felice *et al.*, 1982). Of these isozymes, ALS I is completely insensitive to the sulfonylurea sulfometuron methyl, while ALS II and III are inhibited by this herbicide (LaRossa and Smulski, 1984). The inhibition of ALS II of *S. typhimurium* by sulfometuron methyl exhibits biphasic kinetics, with an initial K_i of 660 ± 60 nM and a final, steady state K_i of 65 ± 25 nM (LaRossa and Schloss, 1984). This slow, tight binding is dependent upon the presence of pyruvate. The inhibition is both reversible and uncompetitive. It is not antagonized by the participants in the enzymatic reaction, i.e., pyruvate, TPP, and FAD. According to Fedtke and Trebst (1986), this suggests an allosteric type of inhibition.

2.2.5 Resistance

In the yeast *Saccharomyces cerevisiae*, 51 spontaneous mutants resistant to sulfometuron methyl have been isolated (Falco and Dumas, 1985). Genetic analysis has demonstrated that these mutations are dominant, tightly linked, and located on the ILV2 gene that encodes acetolactate synthase. An additional 15 sulfometuron methyl-resistant mutants have been isolated. These do not have the mutation occurring on the ILV2 gene, but at two other loci. The complete nucleotide sequences of the wild-type yeast ILV2 gene and a resistant mutation have been determined. The deduced amino acid sequence of the protein indicates a single amino acid change, from proline to serine, in the mutant acetolactate synthase (LaRossa and Falco, 1984).

Several mutants resistant to chlorsulfuron and sulfometuron methyl have been isolated from cultured cells of tobacco (*Nicotiana tabacum*) (Chaleff and Ray, 1984). Plants that were regenerated from these resistant isolates proved to be completely resistant to chlorsulfuron at doses up to 100 times higher than that required to inhibit the growth of susceptible tobacco plants. Using genetic analysis it was demonstrated that resistance could be conferred by a homozygous, single dominant or semi-dominant mutation. Of the six mutations on which linkage analysis was performed, four were on one genetic locus, two on a second locus. All six mutations proved to be true mutations in that resistance was transmitted through sexual crosses in accordance with conventional inheritance patterns. The mutants were resistant to both chlorsulfuron and sulfometuron methyl.

The chlorsulfuron and sulfometuron methyl-resistant tobacco mutants were used to demonstrate that these herbicides inhibit the enzyme acetolactate synthase (Chaleff and Mauvais, 1984). The ALS activity in extracts obtained from normal tobacco cells was inhibited 50% by 14 nM chlorsulfuron. In contrast, 8 μM chlorsulfuron did not cause this level of enzyme inhibition in extracts obtained from resistant tobacco cells. Hence, it appears that an altered enzyme is the basis for the observed resistance.

This was confirmed with chlorsulfuron-resistant mutants of *Arabidopsis thaliana* (Haughn and Somerville, 1986). In this species, resistance is due to a single dominant nuclear mutation at a locus designated *csr1* that has been genetically mapped to chromosome-3. ALS activity in extracts of chlorsulfuron-resistant plants was much less susceptible to inhibition by chlorsulfuron than the activity in extracts of wild-type plants. Recently Haughn *et al.* (1988) have been able to introduce the mutant *Arabidopsis* ALS gene into tobacco plant by a Ti plasmid-mediated transformation. The DNA sequence of the mutant gene differs from that of the wild type by a single base pair substitution, resulting in a proline-to-serine amino acid substitution. The cloned gene conferred over a 100-fold resistance to chlorsulfuron on the tobacco plants.

Saxena and King (1988) have isolated several sulfonylurea-resistant cell lines from haploid cell suspension cultures of *Datura innoxia* P. Mill. These lines showed a high (100- to 1000-fold) resistance to chlorsulfuron and sulfometuron methyl. While some cell lines showed cross-resistance to imidazolinone herbicides (see section 2.3.1), others showed no cross-resistance at all.

2.2.6 Effects on plant metabolism

Treatment of a susceptible plant with chlorsulfuron induces chlorosis, necrosis, epinasty, leaf abscission, anthocyanin accumulation, and stunting, and leads ultimately to the death of the plant. In the period between chlorsulfuron treatment and the death of the plant, several physiological changes can be observed. In velvetleaf seedlings a spray application of 35 g/ha resulted in a stimulation of the production of ethylene, with a maximum occurring 36 h after treatment (Hageman and Behrens, 1984b). In the abscission zone this increase in the production of ethylene occurred simultaneously with an increase in cellulase activity. In sunflower seedlings the application of 10 μg chlorsulfuron to the shoot apical tissue resulted in a stimulation of ethylene production by the cotyledons, the hypocotyls, and the roots. Measuring the ethylene production of hypocotyls excised from chlorsulfuron-treated seedlings, Suttle (1983) found that it reached a maximum 2 to 3 days after treatment. This coincided with an accumulation of ACC[6], a precursor of ethylene. No effect on ethylene production was observed when excised hypocotyls of untreated seedlings were incubated with chlorsulfuron. In soybean seedlings, 1 μg chlorsulfuron applied to the cotyledonary node resulted in an increase in the anthocyanin content of the hypocotyl sections, assessed 8 days later (Suttle and Schreiner, 1981). Similar results were obtained with sunflower seedlings. In this case, an increase in both the total phenolic and hydrocinnamic acid content in hypocotyls excised from treated seedlings was observed (Suttle *et al.*, 1983). It is doubtful that these responses are mediated solely by ethylene. Inhibition of the action of ethylene by silver ions failed to prevent the chlorsulfuron-induced anthocyanin build-up, and exogenous ethylene had no effect on the concentration of phenolic compounds.

---

[6] 1-aminocyclopropane-1-carboxylic acid

2.2.7 Selectivity

The selectivity of chlorsulfuron between tolerant and susceptible species is attributed to differences in the metabolism of the herbicide (See Figure 2) (Hageman and Behrens, 1984a; Hutchinson *et al.*, 1984; Sweetser *et al.*, 1982). In susceptible broadleaf species, little or no metabolism of chlorsulfuron occurs during 24 h after application of the herbicide. In tolerant grass species such as wheat, chlorsulfuron is rapidly metabolized by hydroxylation of the phenyl ring (metabolite A-1), followed by conjugation with a carbohydrate moiety, resulting in a polar, inactive *O*-glycoside of chlorsulfuron (metabolite A). In tolerant broadleaf species such as flax and black nightshade (*Solanum nigrum* L.), detoxification of chlorsulfuron occurs by hydroxylation of the methyl group on the heterocyclic ring (metabolite B-1), followed by conjugation with a carbohydrate moiety (metabolite B).

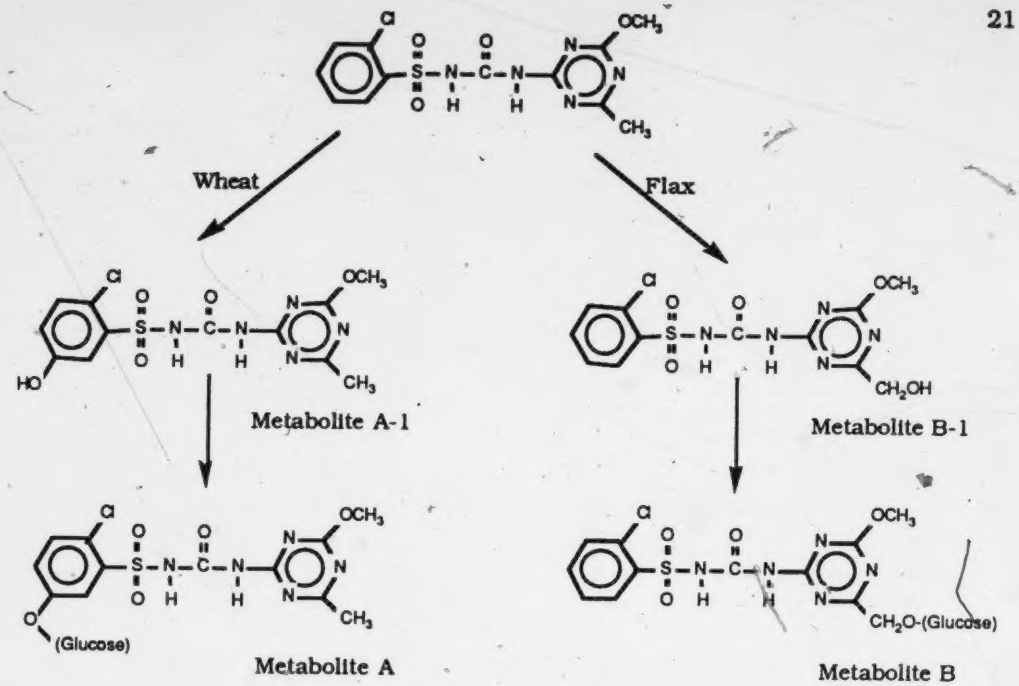


Figure 2. Metabolic pathways of chlorsulfuron in wheat and flax. Metabolites are identified according to references (Hutchinson *et al.*, 1984; Sweetser *et al.*, 1982).

2.3 Other amino acid biosynthesis-inhibiting herbicides

The sulfonylurea herbicides chlorsulfuron and sulfometuron methyl are not the only two herbicides known to inhibit amino acid biosynthesis in plants (See Table 3) (LaRossa and Falco, 1984). Recently it has been reported that the imidazolinone herbicide imazapyr also inhibits the biosynthesis of branched-chain amino acids. The nonselective herbicide glyphosate inhibits the biosynthesis of aromatic amino acids. Aminotriazole has been found to antagonize the biosynthesis of histidine. Phosphinothricin is a competitive inhibitor of glutamine synthase, affecting the biosynthesis of glutamine.

Table 3. Herbicides that inhibit amino acid biosynthesis.

Herbicide	Inhibited pathway	Target enzyme	Apparent K_i (nM)	Source
Chlorsulfuron	Branched chain	Acetolactate synthase	7 21	Tobacco Pea
Sulfometuron methyl	Branched chain	Acetolactate synthase	60 110 16	Bacteria Yeast Pea
Imazapyr	Branched chain	Acetolactate synthase	12,000	Maize
Glyphosate	Aromatic	5-enolpyruvyl-shikimate-3-phosphoric acid synthase	10,000	Bacteria
		3-deoxy-D-arabino-heptulosonate-7-phosphate synthase	500,000	Mung bean
Aminotriazole	Histidine	Imidazole glycerol phosphate dehydratase	30,000	Bacteria
Phosphinothricin	Glutamine	Glutamine synthase	73,000	Pea

2.3.1 Imidazolinones

When corn suspension cells were incubated with 10 µM imazapyr, growth of the cells was inhibited (Anderson and Hibberd, 1985). Accompanying this inhibition was a reduction in the incorporation of radiolabelled amino acids into protein. The inhibition of protein synthesis ranged from 50 to 77%, 48 h after incubation with imazapyr. Incubation of corn suspension culture cells with 1 µM imazapyr for 48 h resulted in an increase in the pool size of most of the free amino acids, except of valine and leucine. Pool sizes of the latter were 52 and 27%, respectively, of the levels found in untreated cell cultures. The effect of the herbicide could be alleviated if the cells were incubated in a medium containing 1 mM of each of the branched-chain amino acids. This suggests that the site of action of imazapyr is one of the steps in the biosynthesis of valine, leucine, and isoleucine.

Subsequently it has been demonstrated that imazapyr inhibits the action of the enzyme ALS (Shaner *et al.*, 1984). Muhitch *et al.* (1987) have isolated and purified (49-fold) this enzyme from corn suspension culture cells. They have demonstrated that imazapyr binds to ALS, both *in vivo* and *in vitro*. Imazapyr is a slow, tight-binding inhibitor of ALS. Initial and final (4 h later) K_i values for the inhibition of the enzyme isolated from maize by imazapyr were 15 and 0.9 μM, respectively. The inhibition appeared to be uncompetitive, suggesting that the herbicide binds to the [enzyme:substrate] complex (Shaner *et al.*, 1984).

Anderson and Hibberd (1985) have reported on a corn line (XA 17) that is resistant to the imidazolinones. The I_{50} values for the inhibition of ALS by the imidazolinones were 1-10 μM in the wild type, and 3-30 mM in the resistant line. This resistant line was also resistant to the sulfonylurea herbicides. For this group the I_{50} values were 0.01 μM and 0.3 μM, respectively (Fedtke ant Trebst, 1986). The absence of cross-resistance to imidazolinones in some *Datura innoxia* cell lines that are resistant to sulfonylurea herbicides suggests that these two groups of herbicides act on slightly different sites on the ALS enzyme (Saxena and King, 1988).

The physiological responses of corn seedlings to an application of imazapyr have been monitored by Shaner and Reider (1986). They found that 24 h after a spray application of 250 g a.e./ha to 21-day old corn seedlings, the level of neutral sugars in the leaves had increased by 39%. Using root tips excised from corn seedlings that had their roots exposed to 150 μM imazapyr, they found that protein synthesis and lipid synthesis were not inhibited 24 h later. During the same period, respiration and RNA synthesis were inhibited by 32 and 15%, respectively, while DNA synthesis was inhibited 70 to 90%. The level of free soluble protein decreased 21%; total free amino acid levels increased 32%. The DNA synthesis inhibition did not begin until 5 to 7 h after the application of imazapyr and could be alleviated by an exogenous supply of valine, leucine, and isoleucine.

2.3.2 Glyphosate

Glyphosate is a potent inhibitor of the shikimic acid pathway (Cole, 1985). This triple-branched pathway is found only in microorganisms and plants, and is initiated by the condensation of phosphoenolpyruvate (PEP) with erythrose-4-phosphate. The major endproducts of this pathway are the aromatic amino acids tyrosine, phenylalanine, and tryptophan. Phenylalanine feeds into secondary phenolic compound pathways via the enzyme phenylalanine ammonia-lyase (PAL, EC 4.3.1.5) to produce such phenolic endproducts as lignin precursors, flavonoids, and tannins.

One of the primary sites of action of glyphosate appears to be the enzyme 5-enolpyruvylshikimate 3-phosphate (EPSP) synthase (E.C. 2.5.1.19) (Amrhein *et al.*, 1982). This enzyme, found predominantly within the plastids (Smart and Amrhein, 1987), catalyzes the formation of 5-enolpyruvylshikimate 3-phosphate from shikimate 3-phosphate and PEP. The inhibition by glyphosate of EPSP synthase extracted from *Nicotiana silvestris* was uncompetitive with respect to shikimate 3-phosphate (Rubin *et al.*, 1984). In the case of EPSP synthase extracted from *Klebsiella pneumoniae* or cultured cells of *Corydalis sempervirens*, the inhibition with respect to shikimate 3-phosphate was noncompetitive (Amrhein *et al.*, 1982). In all systems examined thus far, glyphosate is a competitive inhibitor of EPSP synthase with respect to PEP. The following K_i values for this inhibition have been reported: *Pisum sativum* 0.08 μM (Mousdal and Coggins, 1984), *Klebsiella pneumoniae* 1 μM (Amrhein *et al.*, 1982), *Neurospora crassa* 1.1 μM (Boocock and Coggins, 1983), *Nicotiana* *silvestris* 1.25 μM (Rubin *et al.*, 1984), and *Corydalis sempervirens* 10 μM (Amrhein *et al.*, 1982). These EPSP inhibition patterns imply a mechanism in which glyphosate competes with PEP for binding to an [enzyme:shikimate 3-phosphate] complex. Binding of glyphosate to this complex forms the terminal complex [enzyme:shikimate 3-phosphate:glyphosate]. A glyphosate-resistant carrot (*Daucus carota*) cell line has been reported to have a 5.5-fold increase in EPSP activity and an EPSP protein level of 8.7 times that of the wild type (Hauptman *et al.*, 1988).

Another target for glyphosate appears to be the enzyme 3-deoxy-D-arabinoheptulosonate 7-phosphate (DAHP) synthase (EC 4.1.2.15) (Rubin *et al.*, 1982). This enzyme, the first one in the shikimic acid pathway, catalyzes the condensation of erythrose 4-phosphate and PEP to 7-phospho-2-keto-3-deoxy-D-arabinose-heptonate. In mungbean [*Vigna radiata* (L.) Wilczeck], two isozymes of DAHP synthase have been isolated. The one, stimulated several-fold by Mn^{2+} (DAHP synthase-Mn), is not inhibited by glyphosate; the other, absolutely dependent upon the presence of Co^{2+} for activity (DAHP synthase-Co), is greatly inhibited by glyphosate. This inhibition, which cannot be attributed to Co^{2+} chelation by glyphosate, is competitive with respect to erythrose 4-phosphate.

Attempts have been made to alleviate the growth inhibitory action of glyphosate by supplying the aromatic amino acids tyrosine, phenylalanine, and tryptophan to the organism or tissue. Jaworski (1972) demonstrated that the growth inhibition of *Lemna gibba* and *Rhizobium japonicum* could be overcome in this manner. Although this reversal of growth inhibition has been demonstrated in a wide range of microorganisms and cultured plant cells, only in the case of *Arabidopsis thaliana* has it been demonstrated with intact plants (Amrhein *et al.*, 1980; Gresshoff, 1979; Haderlie *et al.*, 1977; Roisch and Lingens, 1974; Roisch and Lingens, 1980).

Tolerance to glyphosate has been found in plants that overproduced the enzyme EPSP synthase. The glyphosate-tolerant cell line MP4-G of *Petunia hybrida* was shown to overproduce EPSP synthase messenger RNA as a result of a 20-fold amplification of the gene (Shah *et al.*, 1986). In case of tobacco, a mutant gene of the *aroA* locus of *Salmonella typhimurium* was incorporated using the *Agrobacterium tumefaciens* plasmid vector system (Comai *et al.*, 1985). This mutant gene encodes an EPSP synthase with decreased affinity for glyphosate due to a proline-to-serine amino acid substitution. When these transformed tobacco plants were sprayed with glyphosate, their tolerance to glyphosate was correlated with the level of expression of the *aroA* gene. The greater the level of expression, the greater the tolerance of the tobacco to glyphosate.

When plants are treated with glyphosate, one result is an accumulation of shikimic acid in the tissue (Amrhein *et al.*; 1980, Berlin and Witte, 1981). This accumulation of shikimic acid, rather than of shikimic 3-phosphate, has been ascribed to an enzymatic dephosphorylation of the latter. Because the synthesis of aromatic amino acids is inhibited by glyphosate, decreased levels of the free amino acids phenylalanine and tyrosine have been measured following application of the herbicide (Ekanayake *et al.*, 1979; Hoagland *et al.*, 1978; Hoagland *et al.*, 1979; Hollander and Amrhein, 1980; Jaworski, 1972; Nilsson, 1977; Shaner and Lyon, 1980). In contrast, the total amount of free amino acids increased. The result of this deficit of aromatic amino acids is a reduction in the synthesis of protein. In wheat the soluble protein content decreased by 68% after exposure to 0.5 mM glyphosate for 24 h (Cole *et al.*, 1980). No such reduction was observed in maize roots (Duke and Hoagland, 1978) or soybean axes (Hoagland *et al.*, 1979).

2.3.3 Aminotriazole

The herbicide aminotriazole[7] (3-amino-1,2,4-triazole) rapidly inhibits the growth of the bacterium *Salmonella typhimurium* by inhibiting the biosynthesis of histidine (Hilton *et al.*, 1965). *In vitro*, aminotriazole inhibits the activity of the enzyme imidazole glycerol-phosphate dehydratase (EC 4.2.1.19), resulting in the accumulation of the metabolic intermediates imidazole glycerol phosphate and aminoimidazole carboxamide ribotide. The growth inhibition of *S. typhimurium* can be alleviated partly by supplying the organism with histidine. A more complete reversal of growth inhibition was obtained when both histidine and purine were supplied. In higher plants, application of aminotriazole does not result in a decrease in the histidine content or an accumulation of the metabolic intermediates (Hilton, 1969; McWhorter and Hilton, 1967). Therefore, inhibition of histidine biosynthesis does not appear to be a primary site of action of this herbicide in higher plants.

2.3.4 Phosphinothricin

The experimental herbicide phosphinothricin (2-amino-4-methyl-phosphino butyric acid) was initially isolated from a tripeptide (phosphinothrycil-alanyl-alanine) antibiotic produced by *Streptomyces viridochromogenes* (Bayer *et al.*, 1972). This herbicide is a competitive inhibitor of the enzyme glutamine synthetase (EC 6.3.12), the first enzyme involved in the assimilation of ammonia in plants (Leason *et al.*, 1982). The inhibition of glutamine synthetase in higher plants results in an accumulation of ammonia in the tissue. The following K_i values for the inhibition of glutamine synthetase by phosphinothricin have been reported: *Escherichia coli* 0.0059 mM, pea leaf 0.073 mM.

---

[7] Trade name: Amitrole. American Cyanamid Company

3. MATERIALS AND METHODS

3.1 General

3.1.1 Plant material

Stinkweed seedlings were grown from seed[8] in 175-ml styrofoam cups filled with horticultural grade vermiculite. The cups were subirrigated with nutrient solution. The reservoir was a wooden tray (77 by 57 by 6 cm inside dimension) lined with polyethylene and covered with a hardboard lid with 48 holes, sized to accommodate the cups. The plants were grown in a growth cabinet at 23/19 C day/night temperatures with an 18-h photoperiod. Fluorescent lights supplied a photosynthetic photon flux density (PPFD) of 800 $\mu E \cdot m^{-2} \cdot s^{-1}$. The relative humidity was 50%.

In the chlorsulfuron metabolism experiments, flax (cv. Norlin) and wheat (cv. Chinese Spring) seedlings were used also. These seedlings were grown under the same conditions and in the same manner as the stinkweed seedlings.

3.1.2 Nutrient solutions

The nutrient solutions used throughout this study were half-strength Hoagland's solution (Hoagland and Arnon, 1950) modified to contain 1.5 µg/ml iron. In experiments in which L-valine, L-leucine, and L-isoleucine were supplied to the seedlings, the nutrient solution contained 1 mM of each of these compounds.

3.1.3 Herbicide

In all experiments, except the metabolism experiments, chlorsulfuron, ^{14}C-labelled [phenyl-$^{14}C(U)$; sp. act. 152.1 $Bq.nmole^{-1}$; radio-chemical purity 98.9%] or technical product (95% pure)[9], were applied in eight to ten droplets (10 µl total volume) of application solution consisting of 10 mM Na_2HPO_4-citric acid buffer, pH 8.0, containing 10% (v/v) tetrahydrofuran and 0.1% (v/v) surfactant[10] [50% (w/v) octylphenoxypolyethoxyethanol]. Using a Teflon™-coated needle syringe[11], the droplets were placed randomly on the third or fourth true leaf of stinkweed seedlings that had five to seven true leaves. The amount of chlorsulfuron applied varied between experiments and is reported separately for each experiment. Control treatments consisted of eight to ten droplets (10 µl total volume) of application solution without chlorsulfuron.

---

[8] The single source of seed used throughout this study was from a collection made on the University of Alberta Ellerslie Research farm during the summer of 1982.

For the metabolism experiments, ^{14}C-chlorsulfuron was dissolved in 10% (v/v) aqueous ethanol containing 0.5% (v/v) oxysorbic (20POE)[12] (polyoxyethylene sorbitan monolaurate) to a concentration of approximately 1050 Bq per 10 µl solution. A micropipette[13] was used to apply 10 µl herbicide solution as eight to ten droplets to the tip of a young expanded leaf (i.e., the third leaf of wheat or the third or fourth true leaf of stinkweed), or to two expanded leaves near the apex of flax plants that had 20 to 25 leaves.

The technical chlorsulfuron was stored as a 500 µg/ml stock solution in tetrahydrofuran in the dark at -20 C. For each experiment, the chlorsulfuron treatment solutions were prepared freshly by pipetting the appropriate amount of stock solution into a glass microvial, evaporating the solvent, and adding the appropriate amount of application solution. Prior to application, the microvial with the chlorsulfuron solution was held in a sonic bath for 1 min in order to ensure all the chlorsulfuron was dissolved. The ^{14}C-chlorsulfuron was stored dry in the dark at -20 C. Treatment solutions were prepared by dissolving the ^{14}C-chlorsulfuron in tetrahydrofuran and taking an aliquot. This aliquot was used to prepare the treatment solution in the same manner as described above. The solution of ^{14}C-chlorsulfuron in tetrahydrofuran was reduced to dryness again with a stream of nitrogen gas before storage.

---

[9] Technical and ^{14}C-labelled chlorsulfuron were gifts from DuPont, Wilmington, DL.
[10] Citowett Plus, BASF Canada Inc., Calgary, AB.
[11] Unimetrics 5000 series, Terochem Laboratories Inc., Edmonton, AB. Cat. No. TP5010TLC.
[12] Tween 20, Matheson, Coleman and Bell, Manuf. Chemicals, Norwood, OH.
[13] Wiretrol 10 µl, Drummond Scientific Co., Broomall, PA.

3.1.4 Assay procedures

3.1.4.1 Sugar quantification

The assay method for sugars was based on the procedure outlined by Spiro (1966). According to this method, an aliquot of extract or exudate (50 or 75 µl) was combined with anthrone reagent (2 or 3 ml) in a 6-ml culture tube. The tubes were sealed with paraffin film and heated for 10 min at 95 C in an aluminum block. After cooling the tubes in ice, the absorbance at 620 nm was determined. A standard curve, prepared each time with stock solutions of glucose, related the absorbance to the sugar concentration, expressed as mM glucose equivalents.

The anthrone reagent was prepared by combining, in a flask, 400 mg of anthrone with 16 ml of 95% ethanol and 60 ml deionized water. To this solution 200 ml of concentrated H_2SO_4 was added very slowly, keeping the flask cool with running water. The reagent solution was stored in the dark at 4 C.

3.1.4.2 Amino acid quantification

The assay method for amino acids was based on the procedure outlined by Moore (1968). Forty microliters of extract was combined with 360 µl ninhydrin reagent solution[14] in 6-ml culture tubes. The tubes were sealed with paraffin film and heated for 30 min at 90 C in an aluminum block. After letting the tubes cool to room temperature, 1.6 ml water:1-propanol (1:1, v/v) was added. The contents of the tubes were mixed with a vortex mixer and the absorbance at 570 nm was determined. A standard curve, prepared each time with stock solutions of L-leucine, related the absorbance to the amino acid concentration, expressed as mM leucine equivalents. All samples were assayed in duplicate.

---

[14] N 1632. SIGMA Chemical Company, St. Louis, MO, USA.

3.1.4.3 Sample combustion and liquid scintillation spectrometry

Sample combustion was done in a biological sample oxidizer[15] using Carbon-14 cocktail[16] for trapping the $^{14}CO_2$ liberated during the combustion process. For samples that contained both ^3H and ^{14}C, the water containing the ^3H was trapped in Aquasol-2[17]. Sample combustion time ranged between 2 and 4 min, depending upon sample size. When samples with a high ^{14}C-activity were combusted, two vials filled with carbon dioxide-trapping cocktail were used in series.

All radioactivity determinations were done by liquid scintillation spectrometry (LSS)[18], using standard vials. Aqueous aliquots of volumes greater than 150 µl were counted using Aquasol-2 or Ready-Solv™ MP[19] as the cocktail. In all other instances a cocktail consisting of toluene : 2-ethoxyethanol : PPO : POPOP (670 : 330 : 4 : 1, v/v/w/w)[20] was used. Appropriate quench correction curves were generated for each cocktail-aliquot combination used.

---

[15] Harvey OX300. R.J. Harvey Instrument Corp., Hillsdale, NJ.

[16] See address in footnote 15.

[17] NE-952, NEN Research Products, Dupont Canada.

[18] Packard Tri-Carb® 460 CD or Packard Minaxiß Tri-Carb® 4000 Series Liquid Scintillation Counter; Packard Instument Company, Mississauga, Ont.

[19] No. 158728, Beckman Instruments, Inc., Galway, Ireland.

[20] PPO - 2,5-diphenyloxazole
POPOP - 1,4-bis-2-(5-phenoxyloxazoyl)-benzene

3.2 Dose response

Chlorsulfuron, in doses ranging from 0.1 to 5000 ng per plant, was applied to the third leaf of stinkweed seedlings that had four true leaves. Fourteen days later the seedlings were harvested, and shoot height and fresh weight were determined. After drying the shoots for two days at 70 C, the shoot dry weights were determined. The experiment was done twice with four seedlings per dose.

3.3 Growth measurements

In order to monitor the effect of chlorsulfuron on the growth of stinkweed seedlings non-destructively, (a) the daily increases in the shoot weight of individual seedlings were determined, and (b) the rates of elongation of emerging true leaves were measured on a continuous basis.

3.3.1 Increase in shoot fresh weight

Stinkweed seedlings were mounted in pieces of plexiglass (5 x 5 cm), with their roots protruding through a 5-mm diameter hole into nutrient solution. The seedlings were kept in place by means of a piece of paraffin film taped to the underside of the plexiglass. Fresh weights of the shoots were determined daily by placing the seedling-plexiglass assemblies on a wire frame that was mounted on an electronic balance[21] (Figure 3). While on this balance, the roots were hanging in a 125-ml sidearm suction flask filled with nutrient solution. This flask was supported by a laboratory stand and did not touch the balance. Just before taking a reading, the meniscus level of the nutrient solution was adjusted, by means of a syringe attached to the flask, as close as possible to the underside of the plexiglass without touching it. Assuming that the density of the roots approaches that of the nutrient solution, the actual increase in shoot fresh weight over time corresponds to the increase in the weight of the seedling-plexiglass assembly.

---

[21] Mettler AC100

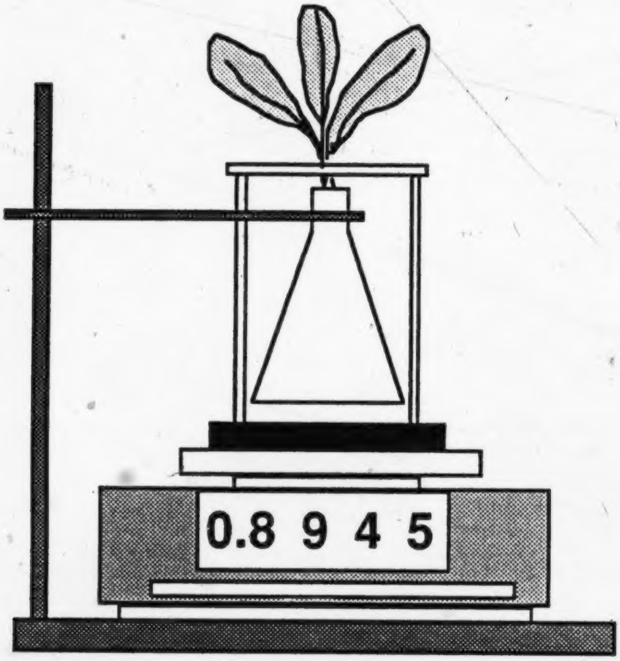


Figure 3. Assembly of flask and balance used to weigh the shoots of stinkweed seedlings.

3.3.2 Rate of leaf elongation

Eight hours before herbicide treatment, the vermiculite was carefully washed off the roots of stinkweed seedlings, and each seedling was mounted with cellulose filler in a piece of plexiglass (10 x 10 cm), with its roots protruding through a 10-mm diameter hole into nutrient solution. The elongation of the fourth true leaf was monitored by means of an angular displacement transducer[22] (Figure 4) that was interfaced with a datalogger[23]. A custom-built sensor arm rested against the tip of the leaf. Due to the symmetrical design of the sensor arm, the force exerted on the leaf was only 130 mg. The datalogger was programmed to record the output signal of the transducer once per minute (1 mV corresponded to 10 μm of vertical displacement of the sensor arm). The data recorded by the datalogger were transferred to a computer by means of a cassette tape, a tape recorder, and a modem[24]. Data were recorded from 2 h before herbicide treatment until 10 h after. All experiments were conducted in a growth cabinet at 20 C, 50% relative humidity, and at a PPFD of 800 $\mu E \cdot m^{-2} \cdot s^{-1}$ supplied by both incandescent and fluorescent lights. For each treatment, measurements were taken on three seedlings.

---

[22] Metripak Transducer, Model 33-03. Gould Inc., Brush Instrument Systems Division, 3631 Perkins Ave., Cleveland, OH.

[23] CR21 micrologger, Campbell Scientific Inc., Logan UT.

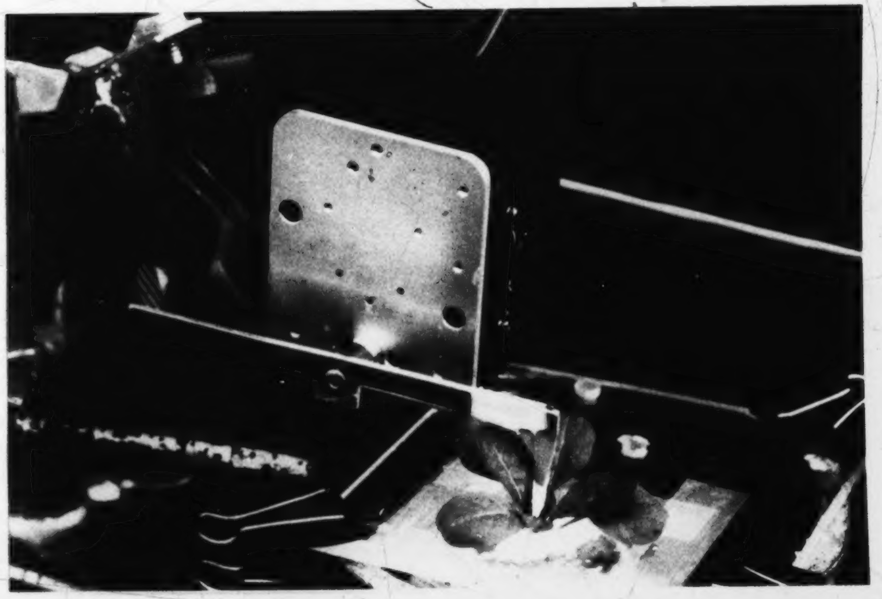


Figure 4. Radial transducer monitoring the rate of leaf elongation of a stinkweed seedling.

---

[24] C20 Cassette Interface, Campbell Scientific Inc., Logan, UT.

3.4 Translocation of chlorsulfuron

Translocation of chlorsulfuron in stinkweed seedlings was assessed by two methods. In the first method, the distribution of ^{14}C-activity among the various parts of the seedlings at different times after application of ^{14}C-chlorsulfuron to a leaf or to the roots was determined. In the second method, a mutilation experiment, the effect of the length of time a chlorsulfuron-treated leaf remained attached to the rest of the seedling, on the dry weight of the shoot 14 days after herbicide treatment, was determined.

3.4.1 ^{14}C-Chlorsulfuron translocation

3.4.1.1 Foliage application

Two series of experiments were conducted. In the first series, stinkweed seedlings were harvested 1, 2, 3, 6, 9, 12, and 24 h after application of 500 Bq of ^{14}C-chlorsulfuron to the third true leaf. The amount of herbicide present on the leaf surfaces at harvest time was determined by rinsing the treated leaf three times with 5 ml 10% (v/v) aqueous ethanol (Devine *et al.*, 1984), dispensed with an automatic pipette[25]. The radioactivity in the rinse solutions was quantified by LSS. The vermiculite was washed off the roots and each seedling was divided into four parts, i.e., roots, treated leaf, shoot apex and the remaining parts of the shoot. The plant parts were stored at -20 C until they were combusted. The experiment was performed twice with three seedlings per treatment.

The second series of experiments was conducted in a manner similar to that described above, except that the radiolabelled chlorsulfuron was applied to the third or the fourth true leaf of stinkweed seedlings. Seedlings were harvested 6, 12, 24, and

---

[25] Finnpipette, 1 to 5 ml adjustable, Labsystems OY, Helsinki, Finland.

48 h after treatment.

3.4.1.2 Root application

Stinkweed seedlings were placed with their roots in 6-ml culture tubes containing 820 Bq of ^{14}C-chlorsulfuron in nutrient solution. The chlorsulfuron concentration in the nutrient solution was 1 μg/ml. The culture tubes were wrapped in aluminum foil and the seedlings were held in place with paraffin film. Throughout the course of the experiments, the nutrient solution level in the tubes was checked every 4 h and, if required, it was adjusted to the original level with deionized water. At harvest time, 1, 2, 3, 6, 12, 24, and 48 h after the start of the experiment, the seedlings were removed from the culture tubes and the roots were dipped in deionized water for 30 sec. The nutrient solution and the water in which the roots were dipped were transferred to scintillation vials and the ^{14}C-activity was assayed by LSS. The roots were blotted dry, weighed, and stored at -20 C. Each shoot was divided into four parts, i.e., apical tissue, third true leaf, fourth true leaf, and remainder of the shoot. The parts were weighed, and stored at -20 C until combustion. The experiment was performed twice with three seedlings per treatment.

3.4.2 Serial mutilation

Stinkweed seedlings were treated by applying 1 μg chlorsulfuron to the third true leaf. The treated leaves were removed at different times after the herbicide application. Fourteen days after the chlorsulfuron treatment, the seedlings were harvested and the dry weight of the shoots was determined after drying at 70 C for 2 days. The experiment was performed twice with six seedlings per treatment.

3.5 Metabolism of chlorsulfuron

In order to determine to what extent stinkweed seedlings metabolize chlorsulfuron, a simple procedure was developed to extract chlorsulfuron and its metabolites from plant tissue that had been treated with ^{14}C-chlorsulfuron.

3.5.1 Method

^{14}C-chlorsulfuron (1050 Bq) was applied to flax, stinkweed, and wheat seedlings (see section 3.1.3). The seedlings were harvested 5 days after treatment. The amount of ^{14}C-activity remaining on the leaf surface was determined according to the method described in section 3.4.4.1. The vermiculite was washed off the roots and the seedlings were wrapped in aluminum foil and stored at -20 C.

3.5.1.1 Extraction procedure

The frozen plant tissue was cut into small sections (10 to 15 mm wide and up to 50 mm long) and ground for 2 min in 25 to 50 ml deionized water in a blender[26] at full speed (Figure 5) (Bestman *et al.*, 1987). The ground tissue was transferred to a 250-ml centrifuge tube and the blender was rinsed with 25 ml deionized water. After centrifugation (10,000*g*, 10 min) the supernatant was filtered into a 900-ml glass jar through Whatman No. 1 filter paper using a 7.5-cm buchner funnel. The pellet and the residue were reground in 25 ml deionized water, the suspension was centrifuged, and the supernatant was filtered into the jar as described previously. The filter paper and pellet (Pellet I, Figure 5) were dried at 60 C and combusted.

---

[26] Model 700A, Waring Products Corp., New York, NY.

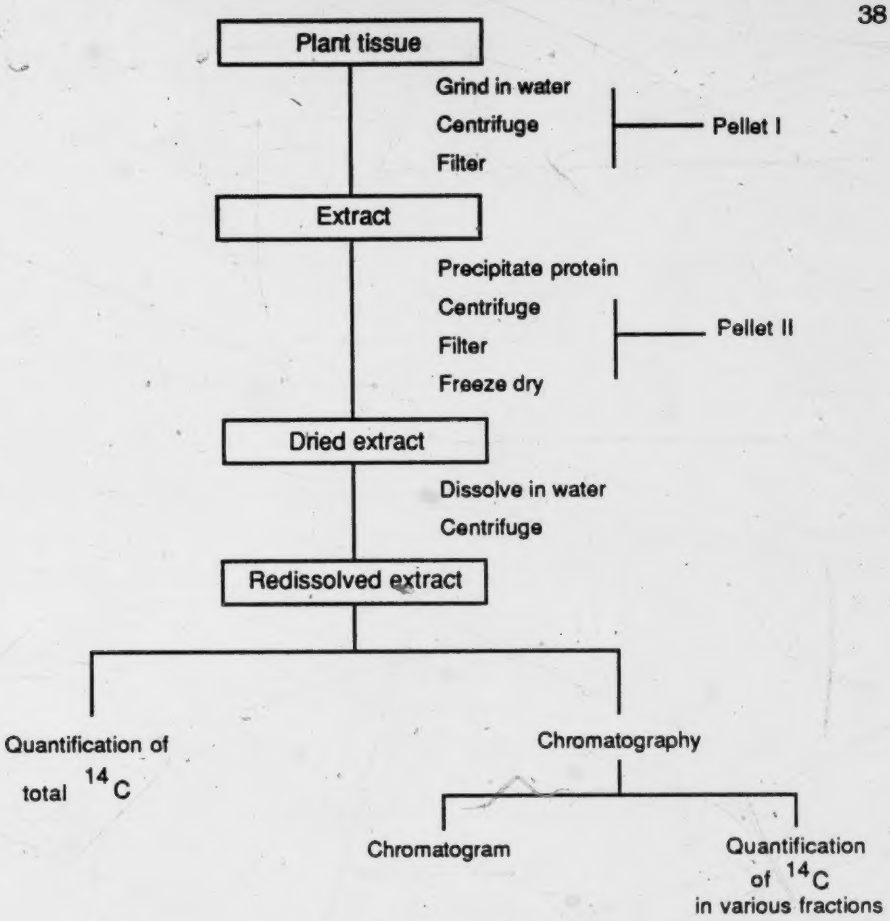


Figure 5. Flow diagram of extraction, separation, and quantification of ^{14}C-chlorsulfuron and its metabolites from plant tissue.

An equal volume of cold (-20 C) acetone was added to the filtrate to precipitate the protein. The jar was covered with paraffin sealing film and stored at 4 C. After a minimum of 12 h, the liquid was transferred to 110-ml glass centrifuge tubes and centrifuged (1000*g*, 10 min). The supernatant was filtered through Whatman No. 1 filter paper directly into a 500- or 1000-ml evaporating flask. The filter paper, together with the pellet (Pellet II, Figure 5), was dried and combusted as described above. The acetone in the supernatant was evaporated at 40 C using a rotary evaporator, and the remaining water fraction was transferred to a freeze-drying flask. The liquid in the flasks was frozen, using a dry ice/acetone mixture, in such a manner as to coat the walls of the flasks.

After freeze-drying, the residue was dissolved in 10 to 20 ml deionized water. The liquid was transferred to a 25-ml pear-shaped boiling flask and reduced to dryness with a rotary evaporator (50 C). The residue was dissolved in 1 ml deionized water and transferred to a 1.5-ml polyethylene centrifuge tube. Twenty microliters of a solution of ^{3}H-sucrose [fructose-1-^{3}H(N); spec. act. 133.2 kBq·nmole^{-1}; radiochemical purity 99.5%][27], containing 830 Bq, was added as an internal standard. The tubes were centrifuged (8,000*g*, 2 min), and a 100-µl aliquot was assayed by LSS. The ratio between the ^{3}H-activity in this aliquot and the amount of ^{3}H-sucrose added initially as the internal standard was used to calculate the total amount of ^{14}C-activity in the extract.

3.5.1.2 Chromatography

A reverse-phase preparative chromatography column[28] was prewetted with 5 ml methanol and flushed with 10 to 15 ml aqueous 0.1% (v/v) formic acid. Using a 250- or 500-µl sample loop, plant extract was loaded on the cartridge and eluted with a step gradient of aqueous 0.1 % (v/v) formic acid and methanol at a flow rate of 0.5 ml/min. A peristaltic pump was used to maintain this flow rate. The steps in the gradient were 0, 10, 25, 35, 45, and 100% methanol. In order to obtain a chromatogram, 2-min fractions were collected directly into liquid scintillation vials, to which 10 ml scintillation liquid was added subsequently. The radioactivity was determined by LSS. For each species a minimum of six plants was used to obtain chromatograms.

---

[27] NET-341, NEN Research Products, Dupont Canada. Lot no. 1060-276.

[28] SEP-PAK C_{18} cartridge, Waters Associates, Milford, MA.

In order to determine the extent of chlorsulfuron metabolism in each species, the total amount of radioactivity eluted by the various methanol concentrations in the step gradient was obtained by collecting the eluent at each methanol concentration in a 50-ml evaporating flask, evaporating the contents to dryness, redissolving it in 1 ml of 45% aqueous (v/v) methanol, and transferring it to a scintillation vial. The experiment was conducted twice, using extracts of four plants of each species that were chromatographed individually, with the activity in each bulked fraction determined separately.

3.5.1.3 Beta-glucosidase assay

In order to determine which fraction contained the *O*-glycoside metabolite of chlorsulfuron, the total eluent at each methanol concentration was collected and reduced to dryness as described above. For each fraction, the residue was dissolved in 1 ml 0.1 M sodium acetate. One 450-µl aliquot was incubated at 35 C for 24 h with 500 µl ß-glucosidase (EC 3.2.1.21, 1010 units·ml^{-1})[29] in 0.1 M sodium acetate (pH 5.5). A second 450-µl aliquot was incubated with 500 µl of 0.1 M sodium acetate. After incubation, 1 ml of cold (-20 C) acetone was added and the samples were stored for a minimum of 12 h at 4 C. Following centrifugation (1000*g*, 15 min), the supernatant was transferred to a 10-ml evaporating flask and reduced to dryness. The residue was dissolved in 200 µl aqueous 0.1 % (v/v) formic acid and chromatographed as outlined above. The experiment was conducted twice, using extracts of four plants per species each time.

---

[29] Calbiochem-Behring, La Jolla, CA. Lot No. 202868

3.5.1.4 Herbicidal activity assay

Extracts of plants of each species were used to determine the herbicidal activity of the various fractions separated by the chromatographic procedure described. Following chromatography, each methanol fraction was reduced to dryness and redissolved in 130 µl of the herbicide application solution described in section 3.1.3. The radioactivity content of a 10-µl aliquot was assayed by LSS. Of each fraction, a total volume of 50 µl was applied in eight to ten droplets to a young, expanded leaf of a stinkweed seedling that had six to eight true leaves. These plants were grown from seed in 450-ml plastic cups filled with an autoclaved (130 C, 138 kPa, 45 min) soil:sand:peat (1:1:1, v/v/v) mixture. The cups were watered daily with care being taken not to wash the applied material off the leaves. Shoot heights and fresh and dry weights were determined 13 days after treatment. The data presented are based on the results obtained with extracts of four plants per species. Each extract was applied to two stinkweed seedlings.

3.5.2 Extent of chlorsulfuron metabolism in stinkweed seedlings

^{14}C-chlorsulfuron (500 Bq) was applied to the third true leaf of stinkweed seedlings. At 12 and 24 h after treatment, the seedlings were harvested. The amount of ^{14}C-activity remaining on the leaf surface was determined according to the method described in section 3.4.1.1. The seedlings then were stored at -20 C. The frozen tissue was extracted (see section 3.5.1.1.), and the extracts were chromatographed (see section 3.5.1.2.). The eluents for each methanol concentration were bulked, reduced to dryness, and assayed for ^{14}C-activity by LSS. The experiment was done twice with three seedlings per treatment.

3.6 CO_2-fixation and assimilate translocation

The effects of chlorsulfuron on CO_2 fixation and assimilate translocation were studied by means of $^{14}CO_2$-pulse labelling experiments conducted in a custom-made assimilation chamber.

3.6.1 Design and construction of assimilation chamber

The assimilation chamber was constructed out of such inert materials as aluminum, brass, glass, stainless steel, and Teflon™. The chamber could accommodate up to six intact stinkweed seedlings in such a manner that the roots, a single leaf of each seedling, and the remaining parts of the shoots were in three completely separate compartments (Figures 6 and 7). Seals between the inner compartment, containing the intact single leaves, and the outer compartment, containing the remaining parts of the shoots, and between the outer compartment and the root compartment, consisted of cellulose filler[30] around the stems and the petioles.

3.6.2 Experimental procedure

Except in the experiments involving excised leaves, all herbicide treatments were applied to seedlings that were mounted in the assimilation chamber 12 to 14 h earlier. Treated seedlings alternated with control seedlings. All herbicide applications were made at 0800 hrs, following a 10-h dark and 2-h light period.

The CO_2-fixation rates of the leaves and shoots in the inner and outer compartment of the assimilation chamber, respectively, were calculated on the basis of the flow rate of the air through the compartments and the difference in CO_2 concentration between the air going into the chamber and the returning air. The concentration of CO_2 in the air was determined by gas-liquid chromatography[31].

---

[30] Polyfilla, Lepage's Limited, Bramalea, Ont.

Hydrocarbon-free air of known CO_2 concentration was supplied independently to both the inner compartment and the outer compartment at a rate that maintained a CO_2 concentration of 400 ± 25 µl/L within each compartment. The roots of the seedlings were placed in individual glass cups containing 15 ml nutrient solution. In order to maintain the air temperature within the chamber at 20±0.5 C, the entire chamber was placed in a temperature-controlled water bath. Both incandescent and fluorescent lights supplied a photosynthetic photon flux density of 300 $\mu E \cdot m^{-2} \cdot s^{-1}$ at the level of the seedlings in the chamber. The relative humidity in the chamber was 40%.

3.6.3 $^{14}CO_2$-labelling procedure

Uniform distribution of $^{14}CO_2$ within the assimilation chamber during the labeling period was obtained by generating $^{14}CO_2$ from [^{14}C]-$NaHCO_3$[32] and lactic acid in an assembly external to the chamber, and circulating it by means of a peristaltic pump through a closed loop connecting the appropriate compartment and the $^{14}CO_2$-generating assembly. During the labeling period, the CO_2 concentration within the compartment was maintained at a near constant level by pumping a sodium bicarbonate solution into the lactic acid in the $^{14}CO_2$-generating assembly at a rate equivalent to the net rate of CO_2 incorporation. In all instances this experimental set-up resulted in more than 95% incorporation of the total amount of ^{14}C-activity supplied. The $^{14}CO_2$ liberated following the labeling period was trapped in CO_2-trapping fluor or in 15% (w/v) KOH.

---

[31] Hewlett Packard 5830A gas chromatograph equipped with a Porapak Q column (80 - 100 mesh) and a thermal conductivity detector, 50 C, helium carrier gas flow 60 ml/min.

[32] Purchased as sterile aqueous solutions in glass ampoules. NEC-086S, NEN Research Products, Dupont Canada. Lot numbers 670-218 and 2156-008.

Figure 6. Assembled assimilation chamber consisting of inner and outer compartment.

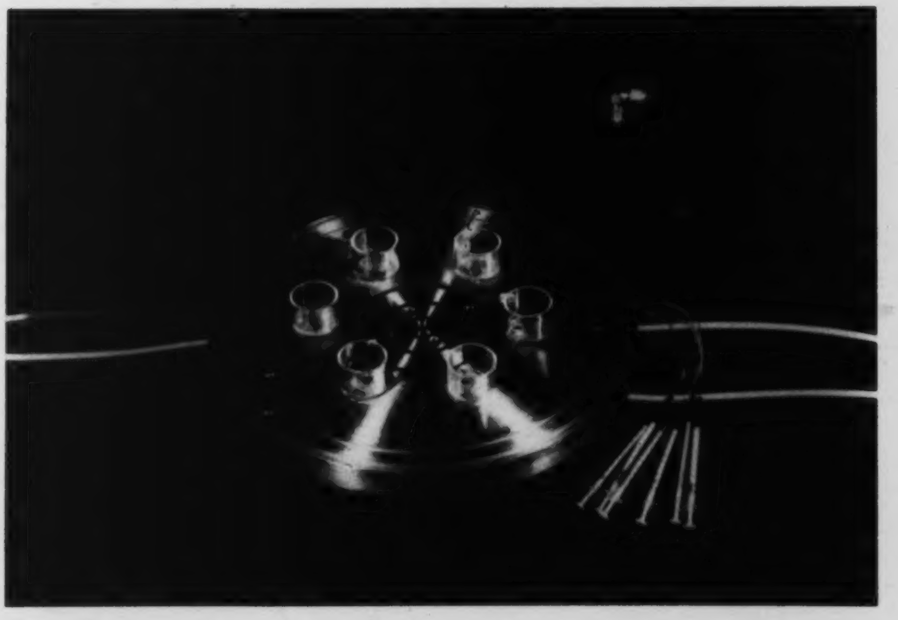


Figure 7. Baseplate of assimilation chamber with the six glass cups for nutrient solution, the syringes connected with Teflon™ lines to each cup, and the Teflon™ air lines for the inner and outer compartment.

3.7 Intact seedling experiments

3.7.1 Incorporation of ^{14}C-activity into various fractions following exposure to $^{14}CO_2$.

Stinkweed seedlings were mounted in the assimilation chamber in such a manner that the third true leaf of each seedling was in the inner compartment (Figure 8). The leaves in the inner compartment were treated with 1 µg chlorsulfuron, and the chamber was closed. At 3, 6, 9, 12, and 24 h after chlorsulfuron treatment, the leaves in the inner compartment were exposed to 740 kBq $^{14}CO_2$ for 30 min. Following a 90-min chase period, the seedlings were harvested by dividing them into four parts, i.e., the $^{14}C0_2$-treated leaf, the shoot apical tissue, the remaining part of the shoot, and the roots. Each part was weighed, and then frozen with liquid nitrogen. All parts were stored in closed vials at -20 C.

Figure 8. Stinkweed seedlings mounted in assimilation chamber. The third true leaf of each seedling is in the inner compartment, the remaining part of each shoot is in the outer compartment.

The apical tissue and the treated leaf were extracted according to the procedures outlined in sections 3.9.1. to 3.9.4. The experiments were done twice with three seedlings per herbicide treatment.

3.7.2 Assimilate translocation

The effect of chlorsulfuron on the translocation of assimilates in stinkweed seedlings was studied (a) in terms of an effect on assimilate translocation out of the herbicide-treated leaf, and (b) in terms of an effect on assimilate translocation out of the leaf adjacent to the herbicide-treated leaf.

3.7.2.1 Chlorsulfuron and $^{14}CO_2$ applied to the same leaf

The experiments were conducted in a manner similar to the ones described in section 3.7.1, except that (a) the leaves in the inner compartment of the assimilation chamber were exposed to $^{14}CO_2$ at 6, 12, and 24 h after herbicide treatment, and (b) all plant parts were combusted. The experiments were done three times with three seedlings per herbicide treatment.

3.7.2.2 Chlorsulfuron and $^{14}CO_2$ applied to adjacent leaves

These experiments were conducted in a manner similar to those described in section 3.7.2.1, except that the fourth true leaf of each seedling was in the inner compartment of the assimilation chamber. The herbicide treatments were applied to the third true leaf in the outer compartment. The leaves in the inner compartment were exposed to $^{14}CO_2$ at 6, 12, and 24 h after the herbicide treatment. The experiments were done three times with three seedlings per herbicide treatment.

3.7.2.3 Uptake of ^{14}C-leucine by the roots and distribution of ^{14}C-activity

The uptake of amino acids by the roots of stinkweed seedlings was studied by supplying ^{14}C-leucine [L-$^{14}C(U)$; sp. act. 12,802 kBq·nmole^{-1}; radiochemical purity

99.7%][33] to the roots and determining the subsequent distribution of ^{14}C-activity within the seedlings. The roots were placed in 6 ml nutrient solution containing 167 kBq of ^{14}C-leucine and 1 mM concentration of each of the amino acids L-valine, L-leucine, and L-isoleucine. The seedlings were harvested 1, 3, 6, 12, or 24 h after the start of the experiment. All plant parts were combusted. The experiment was done twice with three seedlings per treatment.

3.8 Exudation from excised leaves

Experiments with excised leaves were conducted using only the inner compartment of the assimilation chamber. Six 1.5-ml centrifuge tubes were mounted with cellulose filler in the inner compartment of the assimilation chamber. A Teflon™ line ran from each centrifuge tube to the outside of the assimilation chamber, where it was connected to a 5-ml syringe. This design permitted rapid replacement of the exudation solution during the course of an experiment, without opening up the inner compartment.

Phloem exudation from petioles of excised leaves is influenced by the concentration of EDTA in the bathing solution (Groussol *et al.*, 1986; King and Zeevaart, 1974). In order to establish the optimum EDTA concentration, the third or fourth true leaf of stinkweed seedlings was excised, the petioles were recut under water, and the excised leaves were placed with their petioles in individual 1.5-ml centrifuge tubes containing 5 mM phosphate buffer (pH 6.0) with EDTA concentrations ranging from 0.1 to 20 mM. The centrifuge tubes with the leaves were placed in a plexiglass chamber (50 by 28 by 28 cm inside dimensions) within a growth cabinet. Moist air was pumped through the chamber at a rate of 1 L/min. The temperature in the chamber was 25 C, the relative humidity was 60%, and the PPFD at the level of the leaves was 600 $\mu E \cdot m^{-2} \cdot s^{-1}$. At 2, 4, 6, and 8 h after excising the leaves, the bathing solution in the centrifuge tubes was transferred to a culture tube and replaced by fresh solution. The solution in the tubes was freeze-dried and stored at -20 C. The experiment was terminated 8 h after excising the leaves. At this time the area of each leaf[34] and its fresh weight were determined. The experiment was done once with eight leaves per EDTA concentration.

---

[33] NEC-279, NEN Research Products, Dupont Canada. Lot no. 1669-161.

3.8.1 Exudation profiles following chlorsulfuron treatment and exposure to $^{14}CO_2$

3.8.1.1 Chlorsulfuron and $^{14}CO_2$ applied to the same leaf

The third true leaf of stinkweed seedlings was excised 6, 12, or 24 h after treatment with 1 µg chlorsulfuron. The excised leaves were placed in the inner compartment of the assimilation chamber and exposed to 185 kBq of $^{14}CO_2$ for 30 min. At 1, 3, 5, 7, 9, 11, and 13 h after the start of the $^{14}CO_2$ exposure, the bathing solutions in the centrifuge tubes were transferred to culture tubes and replaced by fresh solution. The weight of the solution in the culture tubes was determined before and after taking a 700-µl aliquot. The aliquots were used for ^{14}C-activity assay by LSS. The contents of the culture tubes were freeze-dried. The amounts of reducing sugars in the tubes were determined by the anthrone method described in section 3.1.4.1. The leaves were frozen with liquid nitrogen and stored at -20 C until combustion. The experiments were done twice with three leaves per herbicide treatment.

3.8.1.2 Chlorsulfuron and $^{14}CO_2$ applied to separate leaves

The exudation profiles of leaves adjacent to the chlorsulfuron-treated leaves of stinkweed seedlings were monitored in experiments similar to those described in section 3.8.2.1., except that the fourth true leaf was excised and exposed to $^{14}CO_2$ 24 h after applying the herbicide treatment to the third true leaf.

---

[34] Li-300 Area meter, Li-Cor Inc., Lincoln, NB, U.S.A.

3.8.3 Exudation profiles following chlorsulfuron treatment and application of ^{3}H-sucrose and ^{14}C-sucrose

The third or fourth true leaf of stinkweed seedlings was treated with 1 μg chlorsulfuron. The treatments were applied to a small (1 cm^2), oval-shaped area on the adaxial surface of one half of the leaf. Twelve or 24 h later, the treated leaves were excised and placed with their petioles in 1.5-ml centrifuge tubes containing 5 mM phosphate buffer (pH 6.0) and 0.5 mM EDTA, in the plexiglass chamber described in section 3.8.1. Each leaf was treated with both ^{3}H-sucrose and ^{14}C-sucrose [^{14}C(U); sp. act. 133.2 kBq·nmole^{-1}; radiochemical purity 98%][35]. The application solution was 10% (v/v) aqueous ethanol containing 0.1% (v/v) oxysorbic (20POE) (polyoxyethylene sorbitan monolaurate). The ^{14}C-sucrose was applied to the 1-cm^2 area of the leaf that had been treated with chlorsulfuron or application solution only; the ^{3}H-sucrose was applied to a 1-cm^2 area of the leaf that had not been treated. The two treated areas were located side by side, each on one half of the leaf, an equal distance away from the end of the petiole. The bathing solution was changed 2, 4, 6, 8, 10, and 12 h after the application of radiolabelled sucrose.

Twelve hours after application of the radiolabelled sucrose the leaves were washed with 10% (v/v) aqueous ethanol according to the method of Devine *et al.* (1984). The leaves were frozen with liquid nitrogen and stored at -20 C until combustion. The experiment was done once with six leaves per treatment.

---

[35] NEC-100, NEN Research Products, Dupont Canada. Lot no. 1402-025.

3.8.4 Incorporation of ^{14}C-activity into various fractions following exposure to $^{14}CO_2$

Stinkweed leaves were excised and exposed to $^{14}CO_2$ (185 kBq) 24 h after they had been treated with 1 µg chlorsulfuron. Following a 30-min labelling period and a 330-min chase period, the leaves were weighed, frozen with liquid nitrogen, and stored at -20 C. The leaves were extracted and the extracts were separated and assayed according to the procedures outline in section 3.9.

3.8.5 Incorporation of ^{14}C-activity into soluble leaf proteins following exposure to $^{14}CO_2$

Stinkweed seedlings were transferred to nutrient solution. After 6 to 8 h the third true leaf was treated with 1 µg chlorsulfuron. Twenty four hours after herbicide treatment the leaves were exposed to 1480 kBq $^{14}CO_2$ for 30 min. Following a 330-min chase period the leaves were weighed, frozen with liquid nitrogen, and stored at -20 C. Soluble leaf proteins were extracted according to the procedures outlined in section 3.10. The experiments were done twice with three leaves per herbicide treatment.

3.9 Extraction, separation, and analysis of various fractions from plant tissues

3.9.1 Extraction procedure

The various fractions were extracted according to a procedure similar to that outlined by Dickson (1979). The tissue was homogenized for 1 min in 2 ml methanol:chloroform:water (12:5:3, v/v/v) (MCW) using a glass homogenizer. The liquid was transferred to a centrifuge tube and the homogenizer was rinsed with an additional 2 ml MCW. Following centrifugation (1000*g*, 10 min) the supernatant was transferred to a graduated conical centrifuge tube and the pellet was resuspended in 2 ml MCW. After centrifugation the supernatant was added to the graduated centrifuge tube. The addition of 3.5 ml chloroform and 2.5 ml water, followed by vigorous shaking, vortex mixing (30 sec), and centrifugation (1000*g*, 10 min), resulted in a phase separation. The upper phase, water-alcohol, was transferred to a pearshaped boiling flask and reduced to dryness using a rotary evaporator. The residue was dissolved in 1 ml water, transferred to a 1.5-ml centrifuge tube, and stored at -20 C. The lower phase, chloroform, was transferred to a Teflon™-lined screw cap vial, reduced to dryness with a stream of nitrogen, and stored at -20 C.

3.9.2 Fractionation of water-alcohol fraction

The water-alcohol fraction was further fractionated using an ion exchange chromatography method similar to that of Atkins and Canvin (1971). The whole fraction was loaded on a cation exchange column (4.5 ml; Dowex 50X8-400; hydrogen form) connected in series with an anion exchange column (4.5 ml; Dowex 1X8-400; formate form). With the two columns in series, the neutral fraction, containing the sugars, was eluted with 20 ml water. After disconnecting the columns, the amino acid fraction was eluted from the cation exchange column with 60 ml 3 N HCl. The remaining sugars were eluted from the anion exchange column with an additional 10 ml water after which the acid-1 fraction, containing organic acids, was eluted with 50 ml 6 N formic acid, and the acid-2 fraction, containing 3-phosphoglycerate, ribulosediphosphate, and phosphoenol-pyruvic acid, was eluted with 50 ml 2 N HCl. The cation exchange column was regenerated with 50 ml water. The anion exchange column was regenerated with 65 ml 1 M sodium formate, followed by 65 ml 0.1 N formic acid. The flow rate of the mobile phases through these columns was 1 ml per min. All eluents were collected in pearshaped boiling flasks, and reduced to dryness with a rotary evaporator at 40 C. The residues were dissolved in 1 ml water, transferred to 1.5-ml centrifuge tubes, and stored at -20 C.

3.9.3 Analysis of sugar fraction

The total amount of sugar in this fraction was determined according to the method outlined in section 3.1.4.1. The total amount of ^{14}C-activity was determined by LSS. The individual sugars were separated by HPLC (300 x 7.8 mm Aminex HPX-87H column; ambient temperature; 20 µl sample loop; 0.01 N H_2SO_4 mobile phase; 0.8 ml/min flow rate; refractive index detection). The individual sugars were collected in scintillation vials and the amount of ^{14}C-activity was determined by LSS.

3.9.4 Analysis of amino acid fraction

The total amount of amino acids was determined by the ninhydrin method outlined in section 3.1.4.2. The total amount of ^{14}C-activity was determined by LSS.

3.9.5 Fractionation of lipid fraction

Separation into neutral lipids, galactolipids, and phospholipids was achieved by using silica gel cartridges[36]. The chloroform fraction, 1 ml in volume, was loaded on the cartridge, and the individual lipid classes were eluted successively with 10 ml chloroform, acetone, and methanol, respectively. The eluent of each solvent was collected in a liquid scintillation vial and reduced to dryness using a stream of nitrogen gas. The total amount of ^{14}C-activity in each vial was determined by LSS.

3.9.6 Fractionation of pellet

The pellets were fractionated enzymatically by incubating them with 75 units of pronase (B grade, 45,000 units/g) in 3 ml buffer (0.05 M Tris-H_2SO_4, pH 7.5) for 24 h at 30 C on a wrist-action shaker. Following incubation, centrifugation (1000*g*, 10 min), and washing the remaining pellets twice with water, the supernatants were freeze-dried. The dried residues were dissolved in 5 ml water and the amount of ^{14}C-activity was determined by LSS. The total amount of amino acids, assumed to be indicative of the amount of protein present in the original pellets, was determined by the ninhydrin method. The remaining pellets were combusted.

---

[36] SEP-PAK silica cartridges, Waters Associated, Milford, MA.

3.10 Protein extraction, quantification, and separation

3.10.1 Extraction procedure

The frozen leaf tissue was ground in 3 ml buffer (30 mM Tris-HCl, pH 8.7), containing 6 mg/ml PVP[37], for 30 seconds in a homogenizer[38] (11,000 rpm) and for 3 min in a glass and Teflon™ pestle homogenizer (1000 rpm). The liquid was centrifuged (35,000*g*, 15 min) twice. In each instance, the pellet was transferred to a combustion boat for later combustion. After the addition of 6 ml solution containing 4% (w/v) SDS[39], 5% (v/v) mercaptoethanol, and 5% (w/v) sucrose to the supernatant, the samples were heated to 80 C for 10 min. The proteins were precipitated by the addition of 15 ml cold (-20 C) acetone, storing the samples at 4 C for 15 h, and centrifugation (5,500*g*, 60 min). The pellets were resuspended in 3 ml Tris-HCl buffer (20 mM, pH 8.0).

3.10.2 Protein quantification and specific activity determination

The total amount of protein was determined by the Lowry (1951) procedure. A 0.4 ml aliquot of protein solution was mixed with a solution of 2% (w/v) Na_2CO_3 in 1 N $NaOH^-$: 1% (w/v) sodium tartrate : 0.5% (w/v) $CuSO_4$ (50:1:1, v/v/v). After 10 min at room temperature, 0.2 ml 1 N Folin Ciocalteau reagent was added. The samples were mixed with a vortex mixer and left at room temperature for 30 min before measuring the absorbance at 750 nm. The protein concentration in the samples was calculated using a standard curve prepared with bovine serum albumin. The ^{14}C-activity in the protein solution was determined by LSS. The specific activity was expressed as dpm of ^{14}C-activity per µg of protein.

---

[37] Polyvinylpyrrolidone

[38] Polytron Brinkman Instruments, Rexdale, Ont.

[39] Sodium dodecyl sulfate

3.10.3 Polyacrylamide gel electrophoresis

Soluble leaf proteins were separated using SDS-polyacrylamide gel electrophoresis (SDS-PAGE). Aliquots containing 200 µg protein were pipetted into 1.5-ml centrifuge tubes. After freeze-drying, the protein samples were prepared in 100 µl of solubilizing buffer [50 mM Tris-HCl, pH 7.5, 0.001% (w/v) bromophenol blue, 2% (w/v) sodium dodecyl sulfate (SDS), 20% (v/v) glycerol, 2% (w/v) dithiothreitol] and heated for 5 min at 90 C. The proteins were separated on 7.5-15% (w/v) SDS-polyacrylamide linear slab gels (Chua, 1973) using a 32-cm gel apparatus[40]. The resolving gel contained a 5.1 to 17.2% (w/v) sucrose gradient for stabilization and was buffered with 424 mM Tris-HCl (pH 9.18). The 6% (w/v) stacking gel was buffered with 54 mM Tris-H_2SO_4 (pH 6.1). The buffer in the upper reservoir was 41 mM Tris-H_3BO_3 (pH 8.64) and 0.1% (w/v) SDS; the buffer in the lower reservoir was 424 mM Tris-HCl (pH 9.18). Forty microliters of solubilizing buffer, containing 80 µg protein, was loaded in each lane. SDS-PAGE molecular weight standards[41] included lysozyme (MW 14,400), soybean trypsin inhibitor (MW 21,500), carbonic anhydrase (MW 31,000), ovalbumin (MW 45,000), bovine serum albumin (MW 66,200), and phosphorylase B (MW 92,500). Electrophoresis was carried out at a constant current[42] of 15 mA per gel for 9 h.

---

[40] PROTEAN™ II. Bio-Rad Laboratories (Canada) Ltd., Mississauga, Ont.

[41] Bio-Rad Laboratories (Canada) Ltd., Mississauga, Ont.

[42] Supplied by Electrophoresis Constant Power Supply, ECPS 3000/150, Pharmacia (Canada) Inc., Dorval, Quebec.

The gels were stained for 12 to 14 h in 0.1% (w/v) Coomassie Blue R-250, 25% (v/v) isopropanol, 10% (v/v) acetic acid, and 0.1% (w/v) cupric acetate. The destaining was done in 40% (v/v) methanol and 7% (v/v) acetic acid, followed by soaking in 40% (v/v) methanol, 7% (v/v) acetic acid, and 3% (w/v) glycerol for a minimum of 2 h.

3.10.4 Fluorography

The ^{14}C-activity in the polyacrylamide gels was visualized by means of the fluorographic detection method described by Skinner and Griswold (1983). The gels, after staining and destaining, were soaked in glacial acetic acid for 5 min and in 20% (w/v) PPO[43] in glacial acetic acid for 90 min. Subsequently the gels were soaked in 3% (w/v) glycerol in water for 30 min, after which they were washed liberally with deionized water, and dried at room temperature between cellophane sheets (Wallevik *et al.*, 1982). The dried gels were exposed to preflashed X-ray film[44] at -70 C for 4 weeks.

3.10.5 Protein hydrolysis

Soluble leaf protein was hydrolyzed according to the method of Simpson *et al.* (1976). Five hundred microliters of protein solution was pipetted into 1-ml ampoules and freeze-dried. After the addition of 300 µl 4 N methane sulfonic acid, containing 0.2% (v/v) tryptamine, the ampoules were flame-sealed and incubated for 24 h at 115 C. Following incubation, the ampoules were opened and the CO_2 liberated during the incubation period was trapped in CO_2-trapping fluor. The hydrolysates were partly neutralized by the addition of 700 µl of 5 N NaOH and were fractionated according to the ion exchange chromatography method described in section 3.9.2.

---

[43] 2,5-diphenyloxazole

[44] Kodak Xomat-AR

4. RESULTS AND DISCUSSION

4.1 Response of stinkweed seedlings to increasing doses of chlorsulfuron

The effect of increasing doses of chlorsulfuron on the height, fresh weight, and dry weight of stinkweed seedlings, assessed 14 days after treatment, can be described by a sigmoid log-dose relationship (Figure 9). The equations that describe these relationships were determined according to the method of Streibig (1981; Appendix), and the curves were drawn accordingly. The chlorsulfuron dose at which a 50% inhibition of the respective response parameter occurred (I_{50} value) was resolved graphically. A comparison of the I_{50} values indicated that the height of the stinkweed seedlings was the most sensitive parameter measured. A chlorsulfuron dose of 7 ng/seedling resulted in a 50% reduction in shoot height 14 days later. For fresh weight and dry weight, 134 ng chlorsulfuron per seedling was required to result in a 50% reduction 14 days after application.

4.2 Effect of chlorsulfuron on the growth of stinkweed seedlings

When stinkweed seedlings were treated with 1 μg of chlorsulfuron no visible morphological effects could be observed until 36 to 48 h later. These effects included a stunting of the seedlings and chlorosis of the apical tissue. The growth-inhibiting effect of chlorsulfuron in stinkweed seedlings was measured by monitoring the increase in shoot fresh weight and the elongation of an emerging leaf, immediately following the herbicide treatment.

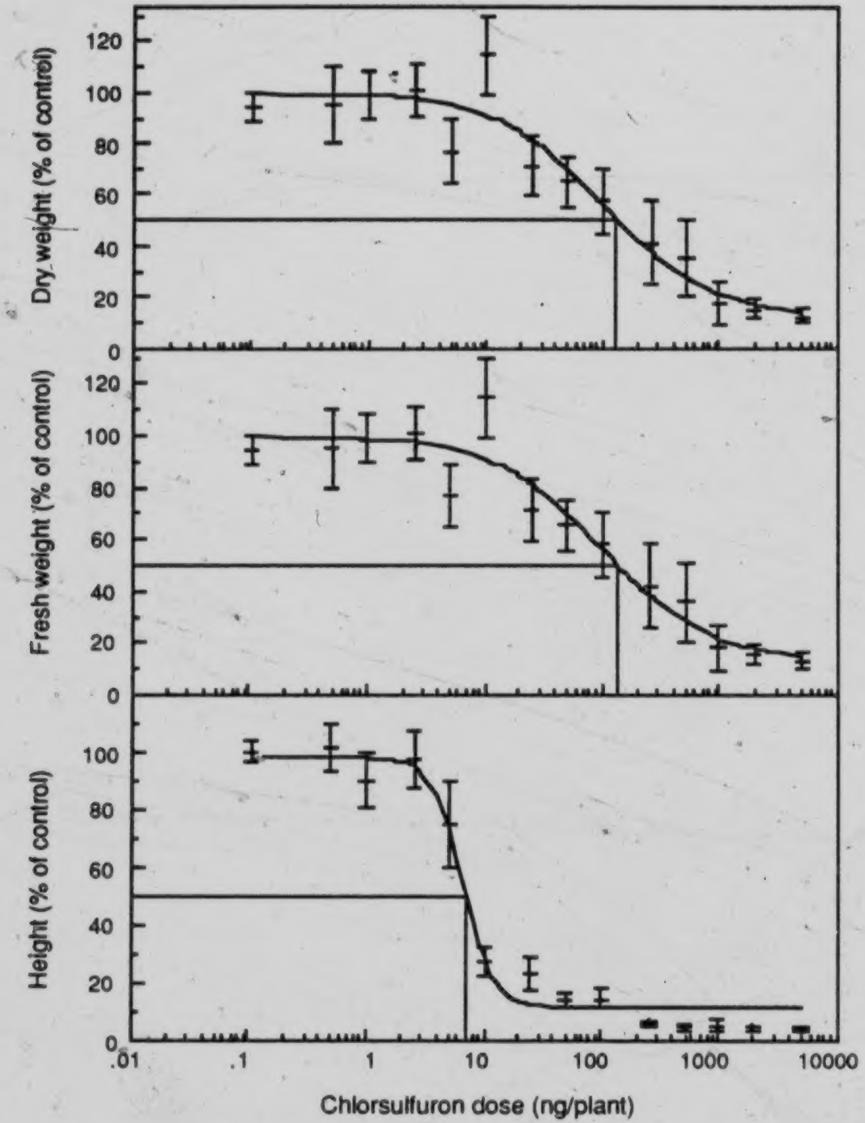


Figure 9. Effect of chlorsulfuron on the height, fresh weight, and dry weight of stinkweed seedlings, 14 days after application to the third true leaf. The data, means and standard errors, are the results of two runs with four seedlings per chlorsulfuron dose, and are expressed as percentages of the response of control seedlings. The sigmoid curve in each graph represents the best fitting relationship (described in appendix, page 122).

4.2.1 Increase in shoot fresh weight

One microgram of chlorsulfuron, applied to the third true leaf of stinkweed seedlings, gradually reduced the rate of growth of the shoot, as indicated by the rate of increase in shoot fresh weight following herbicide application (Figure 10). The average increase in shoot fresh weight of seedlings treated with chlorsulfuron was 32 mg during the 24-h period between 72 and 96 h after treatment, while in the control seedlings the increase during the same period was 72 mg. Between 96 and 120 h after treatment the average increases were 21 and 63 mg, respectively.

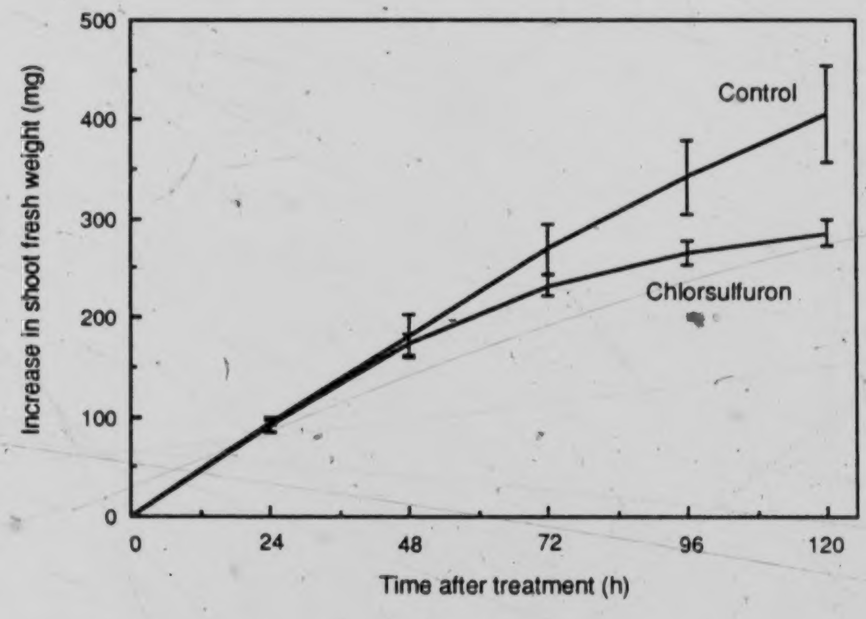


Figure 10. Effect of 1 μg of chlorsulfuron, applied to the third true leaf of stinkweed seedlings, on the increase in shoot fresh weight. The data, means and standard errors, are the results of two runs with four seedlings per treatment.

When, 120 h after chlorsulfuron treatment, the fresh weights of the shoots, as calculated by subtracting the weights of the plexiglass from the weights of the plexiglass/shoot assemblies, were compared with the actual final fresh weights of the shoots, they were, on average, 5.2% higher. This was true for both control and chlorsulfuron-treated seedlings. The discrepancy suggests that the assumption that the density of the roots equals the density of the nutrient solution, is not completely correct. However, the observed discrepancy, although it questions the accuracy of the values obtained for the increases in shoot fresh weights at each time interval, does not invalidate the observation that a foliar application of 1 μg of chlorsulfuron reduced the increase in shoot fresh weight. Especially not because the discrepancy was relatively small and highly consistent.

4.2.2 Rate of elongation of the fifth true leaf

The radial arm transducer was used succesfully to measure the rate of leaf elongation of stinkweed seedlings. For each seedling, the rate of elongation of the fifth leaf during the 2-h period prior to the application of the treatment was considered to be the endogenous growth rate. This growth rate ranged from 0.12 to 0.36 $mm \cdot h^{-1}$. In order to take into consideration the differences in endogenous growth rates and in order to make comparisons between the results of different treatments, the data collected during the 10-h period following application of the treatments are expressed as growth in addition to, or short of, that expected on the basis of linear extrapolation of the endogenous growth rates. The sensor arm of the radial arm transducer described an arc when the leaf elongated but, because of the limited total movement of the sensor arm during the course of a single run, the signal output of the transducer was assumed to be related linearly to the elongation of the leaf.

One microgram of chlorsulfuron, applied to the third true leaf of stinkweed seedlings, had no observable effect on the rates of elongation of the fifth true leaf from the shoot apical tissue during the 10-h observation period following application (data not shown). Measuring the rate of leaf elongation beyond the 10-h period following herbicide treatment was difficult, due to failure of the transducer arm to remain resting on the tip of the elongating leaf.

Different results were obtained when stinkweed seedlings were placed with their roots in nutrient solution containing 1 µg/ml chlorsulfuron (Figure 11). In this instance, a reduction in the rate of elongation of the fifth leaf was observed within 3 to 4 h after placing the seedlings in the solution. This chlorsulfuron-induced reduction in the rates of emergence of the fifth true leaves of stinkweed seedlings could be negated if the nutrient solution containing the chlorsulfuron also contained 1 mM of each of the branched-chain amino acids L-valine, L-leucine, and L-isoleucine (Figure 11). The rate of elongation of the fifth true leaf of stinkweed seedlings placed with their roots in nutrient solution containing the amino acids without chlorsulfuron (Figure 12) was similar to that observed for control seedlings (Figure 13).

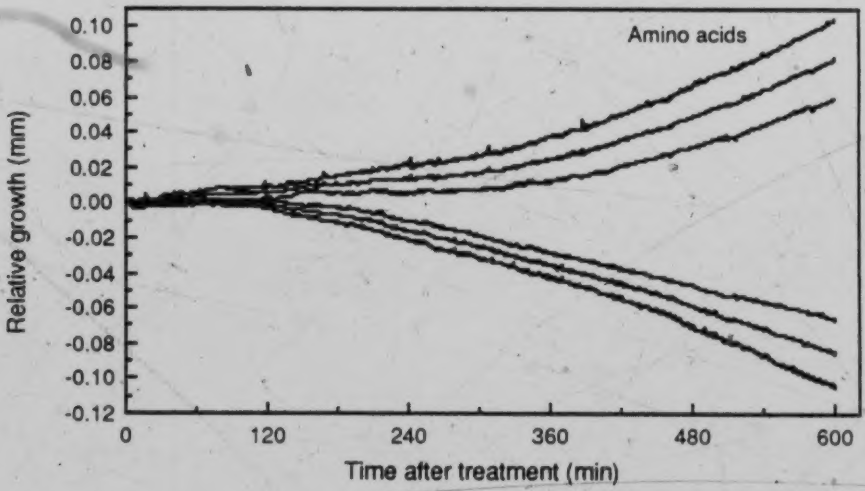


Figure 11. Elongation of the fifth true leaf of stinkweed seedlings growing in nutrient solution containing 1 µg/ml chlorsulfuron, and with (upper set of curves) or without (lower set of curves) 1 mM of each of the amino acids L-valine, L-leucine, and L-isoleucine. The data, means and standard errors, are the results of measurement taken on three seedlings and are expressed as mm of growth in addition to, or short of, that expected on the basis of linear extrapolation of the growth rate of each seedling during the 2-h period before herbicide application.

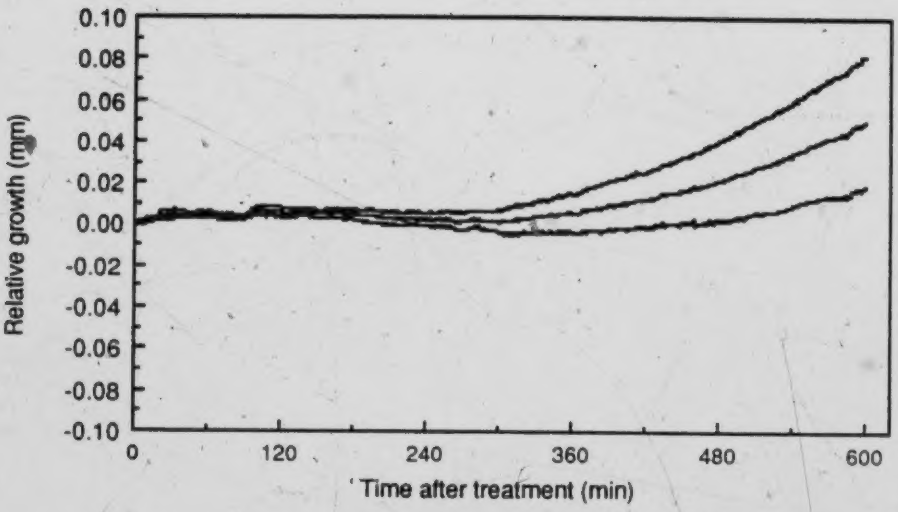


Figure 12. Elongation of the fifth true leaf of stinkweed seedlings growing in nutrient solution containing 1 mM of each of the amino acids L-valine, L-leucine, and L-isoleucine. See Figure 11 for experimental details and description of the data.

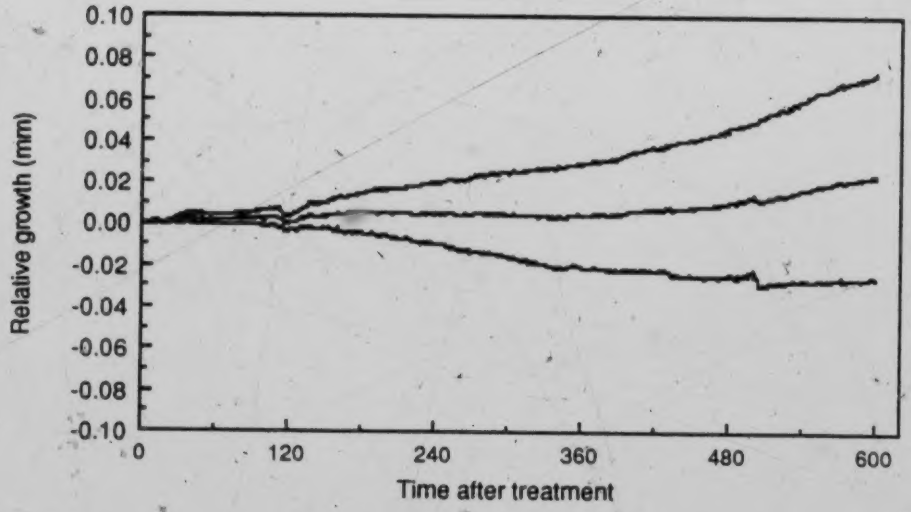


Figure 13. Elongation of the fifth true leaf of stinkweed seedlings growing in nutrient solution. See Figure 11 for experimental details and description of the data.

4.2.3 Discussion

Chlorsulfuron inhibits the growth of stinkweed seedlings. This effect on growth manifests itself especially in terms of the increase in shoot height or the rate of elongation of a new leaf. The effect of the herbicide can be alleviated by supplying the seedlings with branched-chain amino acids. These amino acids, supplied via the roots, appear to counteract the effect of chlorsulfuron, restoring the rate of leaf elongation of chlorsulfuron-treated seedlings to that of control seedlings. No attempt was made to determine for how long the chlorsulfuron-induced effect can be alleviated by the amino acids. In principle it could be a long time, unless the inhibition of the amino acid biosynthetic pathway results in the accumulation of precursors to phytotoxic levels.

These results confirm what has been reported by others regarding the effect of chlorsulfuron on susceptible plants. For example, within 2 h after application, Ray (1982a; 1982b) was able to observe an effect of chlorsulfuron on the rate of elongation of a maize leaf. Within 4 h after application a detectable physiological-biochemical effect could be demonstrated (Ray, 1980). The lack of a measurable effect on the rate of elongation of the fifth leaf of a stinkweed seedling following a 1-µg application to the third leaf, presumably is due to insufficient absorption and/or translocation of the herbicide to the site of action. The rapid effect on leaf elongation following a root application of chlorsulfuron appears to suggest that via this route sufficient chlorsulfuron is absorbed and then translocated to the shoot apical tissue.

The choice of a foliar application dose of 1 µg/plant was based on considerations other than the dose-response relationship. In experiments involving ^{14}C-chlorsulfuron, a minimum foliar application of 500 Bq per plant was deemed to be required in order to have sufficient radioactivity in the plant samples for assaying purposes. Based on the specific activity of the ^{14}C-chlorsulfuron, 500 Bq is equivalent to 1 μg of chlorsulfuron. Although 1 μg of chlorsulfuron per stinkweed seedling appears to be more than what is required to produce a phytotoxic effect, for comparison purposes it was used throughout this study.

4.3 Absorption and translocation of chlorsulfuron in stinkweed seedlings

4.3.1 Foliar application

Chlorsulfuron is absorbed by the leaves of stinkweed seedlings. At 12 h after the application of ^{14}C-chlorsulfuron to the third true leaf, 7.8% of the recovered ^{14}C-activity had been absorbed; at 24 h this had increased to 25.2% (Figure 14). Similar results were obtained, in another series of experiments, when ^{14}C-chlorsulfuron was applied to the third or the fourth true leaf (Figure 15). In this case the average absorption at 12 h was 8.3%; at 24 h this had increased to 12.5%; at 48 h to 30.0% of the total recovered activity.

Chlorsulfuron is translocated from the site of application to the other parts of stinkweed seedlings. When ^{14}C-chlorsulfuron was applied to the third true leaf, 1.7% of the total ^{14}C-activity recovered from the tissue 24 h later was recovered from parts other than the treated leaf (Figure 14). Figure 15 presents the distribution of ^{14}C-activity following application of ^{14}C-chlorsulfuron to the third or the fourth true leaf. Several comments can be made about these results. Chlorsulfuron is translocated out of the treated leaf to other parts of the stinkweed seedlings. Application of chlorsulfuron to the third or fourth true leaf resulted in similar distribution patterns. The difference in physiological age or location on the shoot between the third and fourth true leaf does not seem to affect the distribution pattern. Very little chlorsulfuron is translocated to the roots or the shoot apical tissue. The large standard errors are indicative of the high variability encountered in these experiments. Part of this variability can be attributed to the low specific activity of chlorsulfuron (See section 4.2.3). The total amounts of radioactivity recovered in the apical tissue or the roots were very small, making accurate estimation difficult.

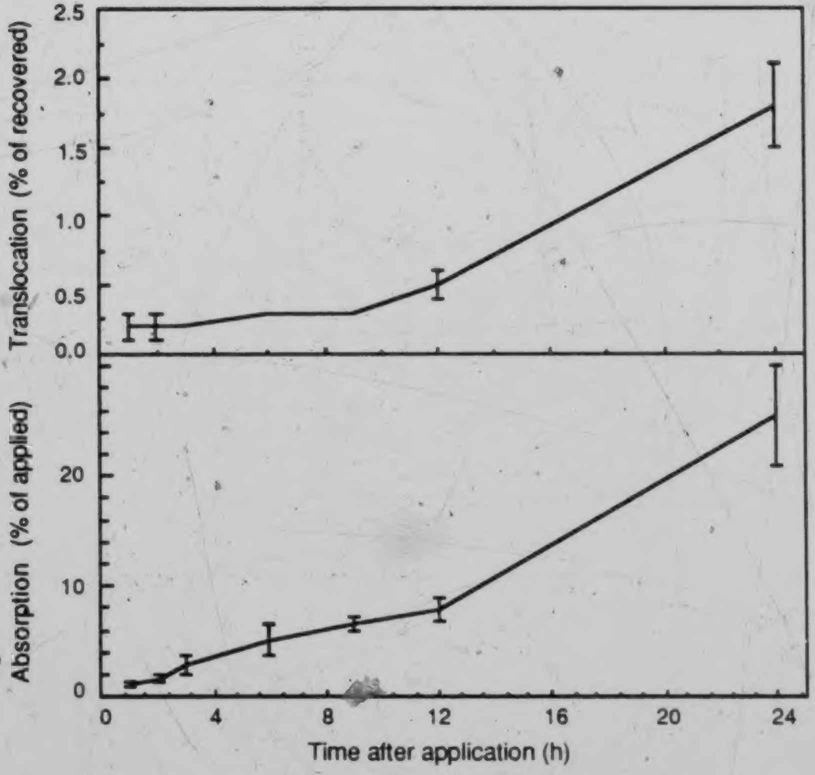


Figure 14. Absorption and translocation of ^{14}C-chlorsulfuron in stinkweed seedlings. The data, means and standard errors, are the results of two runs with three seedlings per treatment.

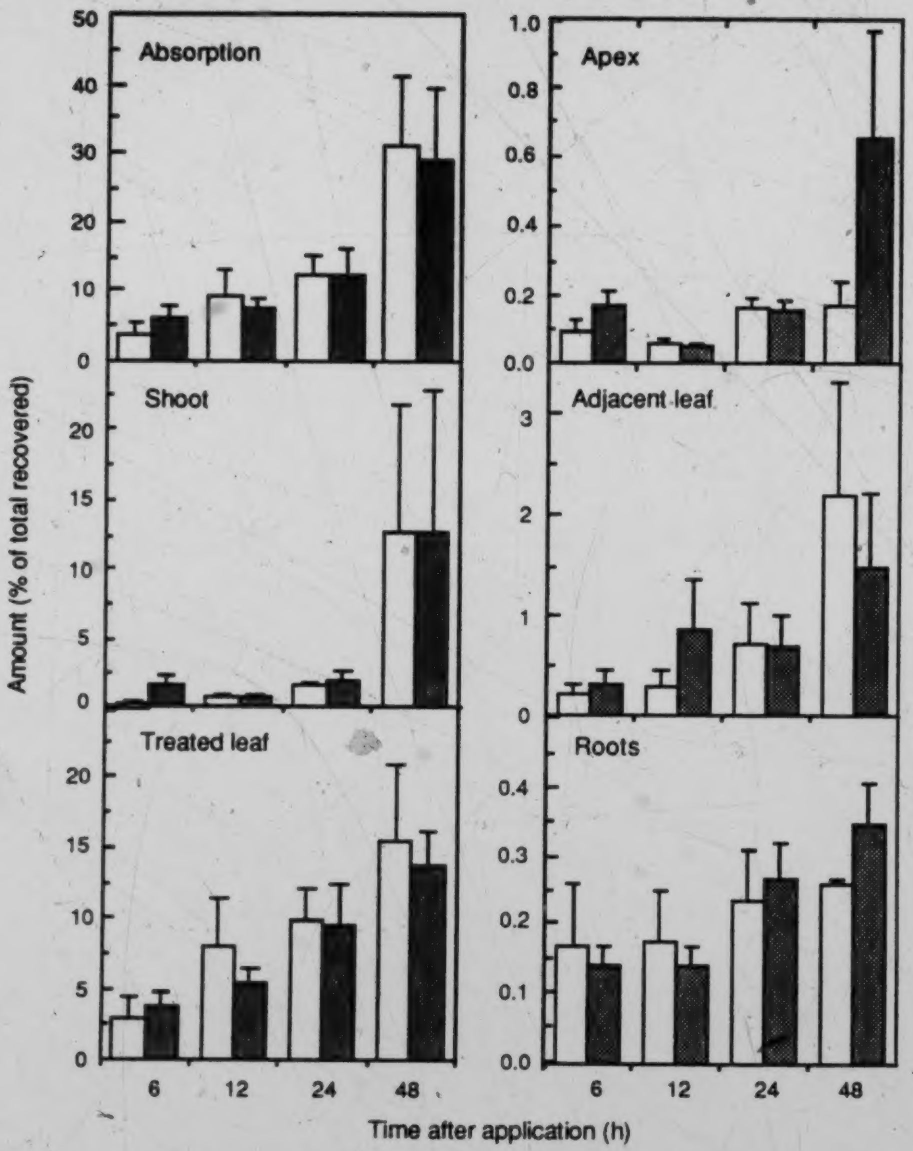


Figure 15. Distribution of ^{14}C-activity in stinkweed seedlings following application of ^{14}C-chlorsulfuron to the third (□) or fourth (■) true leaf. The data, means and standard errors, are expressed as percentages of the total amount of ^{14}C-activity recovered, and are the results of two runs with three seedlings per treatment. In the case of ^{14}C-chlorsulfuron application to the third leaf, the adjacent leaf was the fourth leaf, and vice versa.

4.3.2 Root application

Chlorsulfuron was absorbed by the roots of stinkweed seedlings and was translocated to the various parts of the shoot (Figure 16). The rate of uptake of chlorsulfuron by the seedlings was almost linear over time. Very little chlorsulfuron remained in the roots.

4.3.3 Comparison between foliar and root application

In order to facilitate comparisons between the different types of absorption and translocation experiments, all the amounts of chlorsulfuron absorbed, translocated, or recovered were expressed as picomoles of herbicide (Tables 4 and 5). There is very good correspondence in absorption 6 and 12 h after treatment between the results of experiments in which chlorsulfuron was applied to the third true leaf only and the ones in which it was applied to the third or fourth true leaf. This similarity in results [illegible] rather remarkable since the experiments were conducted two years apart. The data for 24 h after application do not agree very well.

In terms of the amount of chlorsulfuron translocated out of the treated leaf, the results of the two experiments do not agree with each other very well. In the second experiment more herbicide was translocated out of the treated leaf than in the first one. No obvious explanation for this difference can be provided. The conditions under which the seedlings were grown were similar in both experiments and the seedlings had the same physiological age at the time of herbicide treatment.

After a root application of chlorsulfuron the total amount of herbicide in the stinkweed seedlings was higher than after an application to the foliage. For example, 12 h after application the amount of chlorsulfuron in the stinkweed seedlings was approximately twelve times greater following root application than following a foliar application.

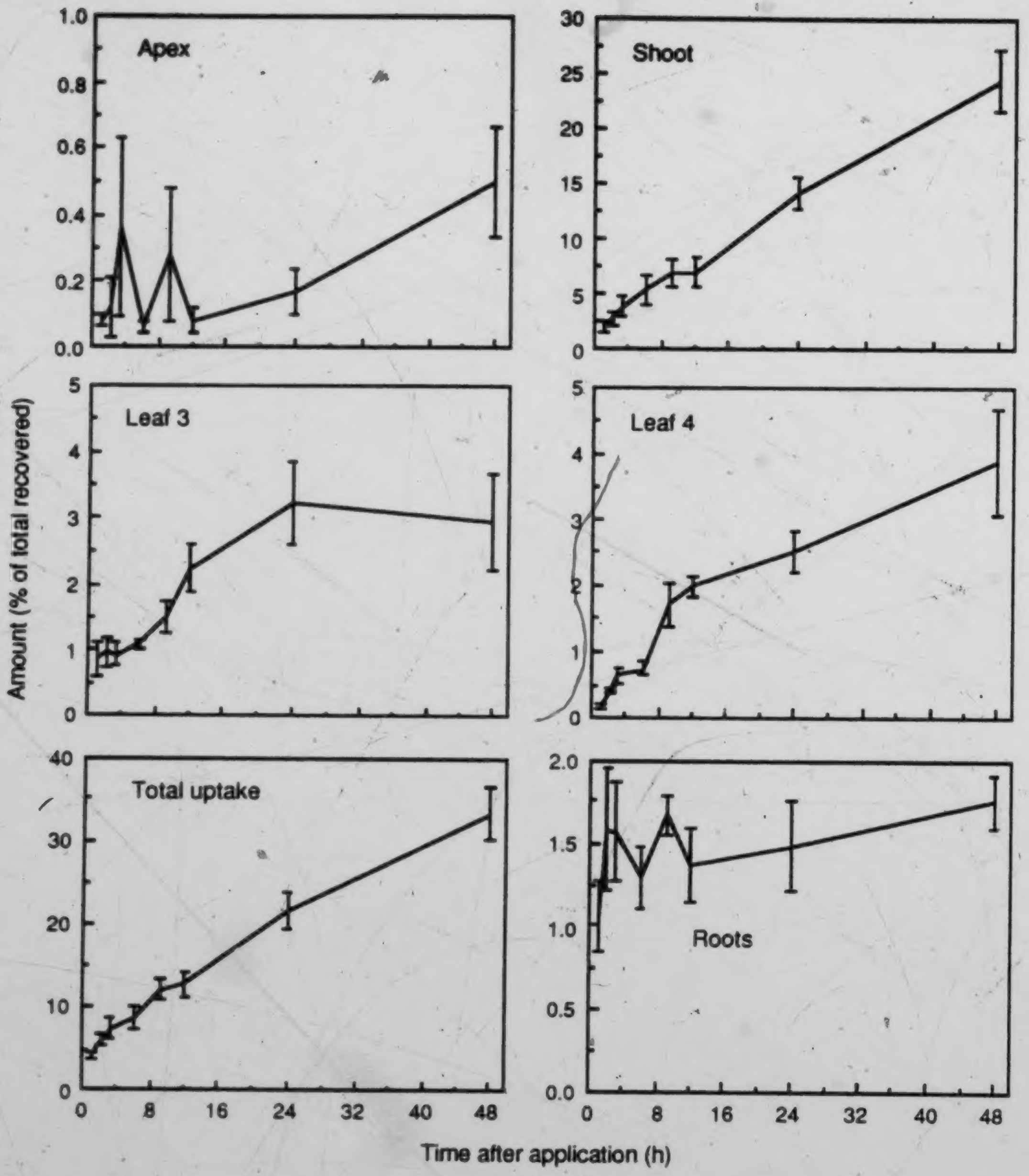


Figure 16. Accumulation of chlorsulfuron in stinkweed seedlings via uptake from nutrient solution containing 1 µg/ml chlorsulfuron. The data, means and standard errors, are the results of two runs with three seedlings per treatment.

Table 4. **Absorption and translocation of chlorsulfuron in stinkweed following application to the foliage or the roots. The data (means with standard errors in parentheses) are expressed as picomoles of herbicide absorbed and translocated, and are the combined results of three series of experiments with ^{14}C-chlorsulfuron.**

	Absorption			Translocation	
	Application[a]			Application[a]	
Time	Leaf(3)	Leaf(3/4)	Root	Leaf(3)	Leaf(3/4)
(h)	(picomoles)				
1	32.7 (6)		711 (76)	6.8 (2.6)	
2	46.2 (6)		1010 (100)	4.7 (1.2)	
3	81.8 (23)		1243 (197)	7.2 (1.3)	
6	142 (38)	140 (32)	1459 (233)	7.2 (1.2)	
9	181 (16)		2032 (211)	9.4 (1.4)	
12	218 (31)	230 (51)	2760 (651)	13.8 (2.4)	45.3 (10.6)
24	707 (125)	349 (63)	3632 (365)	48.2 (7.6)	79.4 (15.3)
48		840 (193)	7755 (1624)		428 (191)

[a] Leaf(3) refers to a series of experiments in which ^{14}C-chlorsulfuron was applied to the third true leaf; leaf(3/4) refers to application to the third or the fourth true leaf; root refers to an application of chlorsulfuron to the roots.

There was fair agreement in the concentrations of chlorsulfuron in the treated leaf between the two series of foliage-treatment experiments. The concentration of chlorsulfuron in the third true leaf following a root application was considerably higher than following a foliar application. Chlorsulfuron did accumulate slowly in the adjacent leaves following a foliar application. There was very little difference in chlorsulfuron concentration in the third or the fourth true leaf following an application of the herbicide to the roots.

Table 5a. Accumulation of chlorsulfuron in stinkweed seedlings following application to the foliage or to the roots. The data (means with standard errors ion parentheses) are expressed as picomoles of herbicide per mg of fresh weight of the tissue, and are the combined results of three series of experiments with ^{14}C-chlorsulfuron.

	Treated leaf or leaf # 3 (after root application)			Adjacent leaf	
Time	Application[a]			Application[a]	
	Leaf(3)	Leaf(3/4)	Root	Leaf(3)	Leaf(3/4)
(h)	(picomoles/mg)				
1	0.20 (0.04)		1.24 (0.40)		0.26 (0.05)
2	0.35 (0.04)		1.27 (0.32)		0.62 (0.07)
3	0.70 (0.22)		1.25 (0.22)		1.14 (0.34)
6	1.19 (0.33)	0.98 (0.23)	1.69 (0.21)	0.09 (0.02)	1.31 (0.21)
9	1.32 (0.17)		2.12 (0.46)		2.16 (0.20)
12	1.39 (0.21)	2.12 (0.60)	4.57 (1.05)	0.21 (0.09)	4.39 (0.87)
24	4.21 (0.77)	2.79 (0.57)	5.13 (1.36)	0.24 (0.08)	4.03 (0.96)
48		3.69 (0.87)	6.20 (2.04)	0.46 (0.15)	11.11 (5.60)

[a] Leaf(3) refers to a series of experiments in which ^{14}C-chlorsulfuron was applied to the third true leaf; leaf(3/4) refers to application to the third or the fourth true leaf; root refers to an application of chlorsulfuron to the roots.

The concentration of chlorsulfuron in the remaining part of the shoot following foliar application was very low, just above detection level. Much more (100-400 times) chlorsulfuron accumulated in the shoot following root application. Similarly, in the shoot apical tissue and in the roots, more chlorsulfuron accumulated after an application to the roots than after a foliar application.

Table 5b. (cont.)

Time	Shoot			Shoot apex			Roots		
	Application[a]			Application[a]			Application[a]		
	Leaf (3)	Leaf (3/4)	Root	Leaf (3)	Leaf (3/4)	Root	Leaf (3)	Leaf (3/4)	Root
(h)	(picomoles/mg)								
1	0.01 (0.00)		0.84 (0.23)	0.03 (0.02)		1.35 (0.29)	0.00 (0.00)		0.58 (0.12)
2	0.01 (0.00)		0.95 (0.25)	0.03 (0.03)		3.07 (2.45)	0.00 (0.00)		0.67 (0.00)
3	0.01 (0.00)		1.24 (0.33)	0.03 (0.02)		7.07 (5.00)	0.00 (0.00)		0.67 (0.07)
6	0.01 (0.00)	0.07 (0.04)	2.05 (0.47)	0.04 (0.03)	0.51 (0.10)	0.99 (0.23)	0.01 (0.00)	0.03 (0.01)	0.66 (0.08)
9	0.01 (0.00)		2.20 (0.36)	0.04 (0.03)		5.41 (3.70)	0.01 (0.01)		0.75 (0.10)
12	0.01 (0.00)	0.06 (0.01)	3.97 (1.18)	0.02 (0.02)	0.19 (0.02)	1.21 (0.38)	0.02 (0.01)	0.04 (0.01)	1.38 (0.63)
24	0.04 (0.01)	0.13 (0.03)	5.22 (0.91)	0.04 (0.03)	0.58 (0.12)	3.15 (1.09)	0.04 (0.01)	0.06 (0.01)	0.86 (0.15)
48		0.98 (0.54)	9.26 (2.10)		1.34 (0.55)	7.86 (2.30)		0.09 (0.01)	1.52 (0.52)

[a] Leaf(3) refers to a series of experiments in which ^{14}C-chlorsulfuron was applied to the third true leaf; leaf(3/4) refers to application to the third or the fourth true leaf; root refers to an application of chlorsulfuron to the roots.

4.3.3 Mutilation study

Another indication of the translocation characteristics of chlorsulfuron was obtained by conducting a serial mutilation experiment. The results of such an experiment (Table 6) suggest that during 4 h after treatment enough chlorsulfuron was exported from the treated leaf to result in a reduction in shoot dry weight 14 days later. In other words, at 4 h after treatment, sufficient herbicide had translocated to the remainder of the plant to cause an effect on growth.

Table 6. The effect of removal of the treated leaf of stinkweed seedlings at different times after an application of 1 μg chlorsulfuron, on the increase in shoot dry weight, assessed 14 days after herbicide treatment. Data are based on two runs with six plants per treatment, and are expressed as percentages of the increase in the pooled control treatments.

Time of shoot removal	Increase in shoot dry weight: Mean	Increase in shoot dry weight: Standard error	T-probability[a]
(HAT[b])	(% of pooled control seedlings)		
0.0	113	11.4	
0.5	124	10.3	0.488
1	125	11.5	0.393
2	96	12.0	0.209
4	56	12.7	0.000
8	50	7.3	0.000
12	37	5.6	0.000
24	25	3.3	0.000

[a] The T-probability is based on a single degree of freedom comparison with the increase in shoot dry weight when the treated leaf was removed at treatment time.
[b] Hours after treatment.

4.3.4 Discussion

The results confirm that chlorsulfuron is absorbed by the roots and foliage of stinkweed seedlings. However, the amounts that were absorbed following a foliar application are considerably smaller then the amounts reported for seedlings of other species (Sweetser *et al.*, 1982; Hageman and Behrens, 1984a). This discrepancy can be due to differences in the methods used to assess absorption of the herbicide, or to inherent differences between the species or plants used. Sweetser *et al.* (1982) used acetone to remove unabsorbed herbicide from the leaf surface of sugarbeet, soybean, mustard, cotton, wheat, barley, and wild oat seedlings. Hageman and Behrens (1984a) used an aqueous solution of 0.01% (v/v) Triton X-100 to wash unabsorbed chlorsulfuron from the leaves of eastern black nightshade and velvetleaf seedlings. Based on the physical-chemical properties of chlorsulfuron (Table 1) it is not expected that the use of either of these wash solutions would overestimate absorption. The triple rinse technique with 10% (v/v) aqueous ethanol used in this study has been shown to be effective in removing all of the unabsorbed chlorsulfuron from the leaf surface of Tartary buckwheat, Canada thistle, and barley seedlings (Devine *et al.*, 1984). Hence, it must be assumed that the seedlings used in this study absorbed less chlorsulfuron than has been reported for seedlings of other species. This difference might be due to inherent differences in the cuticles or to differences in the conditions under which the seedlings were grown. The seedlings used in this study might have had a thicker cuticle due to the high light intensity and the low relative humidity under which they were grown.

Chlorsulfuron was translocated out of the treated leaf or roots to other parts of stinkweed seedlings. Translocation out of the roots suggests that the herbicide is mobile in the xylem; translocation out of the treated leaf indicates that it is also mobile in the phloem. The accumulation of chlorsulfuron in the leaf adjacent to the treated leaf suggests that chlorsulfuron can be transferred from the phloem to the xylem. Presumably the herbicide was exported from the treated leaf via the phloem and, because of reduced phloem transport into the adjacent leaf (see section 4.5.2), largely imported via the xylem into this leaf. The percentage of the absorbed chlorsulfuron translocated out of the treated leaf of stinkweed seedlings is considerably less than has been reported for other seedlings (Hageman and Behrens, 1984a).

The comparatively low phloem mobility of chlorsulfuron is difficult to explain on the basis of its physical-chemical properties. Clopyralid[45] (3,6-dichloropyridinecarboxylic acid), a herbicide with comparable pK_a value but lower lipophilicity than chlorsulfuron, is much more mobile. In some species almost all of the clopyralid that is absorbed by a mature leaf can be exported from that leaf (Devine and Vanden Born, 1985). Devine *et al.* (1987) demonstrated that, on the basis of their accumulation into excised tissue and subsequent elution, chlorsulfuron should be as mobile or even more mobile than clopyralid in the phloem. Apparently, accumulation of a herbicide into excised tissue is not necessarily indicative of phloem mobility of that herbicide.

The results of the absorption and translocation experiments can provide an explanation for the failure to detect an effect of chlorsulfuron on elongation of the fifth true leaf of stinkweed seedlings following an application of chlorsulfuron to the third true leaf (section 4.2.2). The concentration of chlorsulfuron in the shoot apical tissue following a foliar application was considerably less than following an application to the roots. Assuming that the concentration of chlorsulfuron in the shoot apical tissue is a key factor in causing the observed effect on leaf elongation, the concentration following a foliar application presumably was too low to induce this effect.

4.4 Metabolism of chlorsulfuron

When studying the mode of action of a herbicide it is important to know if the phytotoxic effect is caused by the nonmetabolized herbicide molecules or by one or more of its metabolic products. As discussed in section 2.2.7, in the case of chlorsulfuron it is known that the nonmetabolized molecules exert the phytotoxic effects; the metabolic products are not phytotoxic. In order to ascertain if this is also true for stinkweed seedlings, the metabolism of chlorsulfuron in this species was studied.

---

[45] Marketed under the tradename LONTREL.

4.4.1 Procedure

Published methods for the extraction of chlorsulfuron and its metabolites from plant tissue involve the use of organic solvents, solvent partitioning, and separation by means of HPLC or TLC. The former separation procedure limits itself to laboratories equipped with an HPLC unit, while with the latter procedure quantification is cumbersome. Problems encountered following these procedures include (a) inadequate separation of chlorsulfuron and its metabolites during solvent partitioning, and (b) a final extract that contains substantial amounts of plant pigments. The procedure reported here is an alternate procedure (Bestman *et al.*, 1987).

4.4.1.1 Extraction

On average, 77% of the applied ^{14}C-chlorsulfuron (90% of the total activity recovered) was absorbed by the three species 5 days after treatment (Table 7). This compares favourably with data reported for the absorption of chlorsulfuron by Canada thistle leaves (Devine and Vanden Born, 1985).

Averaged across the three species, 94% of the absorbed ^{14}C-activity was recovered in the aqueous extract (Table 7). This is considerably higher than has been reported for the extraction of chlorsulfuron from 'spiked' green plant material using an organic solvent-based procedure (Slates, 1983). The remaining 6% was distributed among pellet I (4%) and pellet II (2%). The total recovery averaged 85% of the applied activity, which is in close agreement with previously reported recovery data for Canada thistle (Devine and Vanden Born, 1985).

Table 7. Distribution of ^{14}C-activity recovered from flax, stinkweed, and wheat treated with ^{14}C-chlorsulfuron [a,b].

Description	^{14}C-distribution					
	Flax		Stinkweed		Wheat	
	(% of applied)					
Leaf wash	4.2	(1.2)	11.9	(5.2)	10.1	(3.1)
Pellet I	3.6	(0.7)	3.0	(1.5)	2.2	(0.4)
Pellet II	1.4	(0.2)	1.7	(0.2)	1.2	(0.1)
Extract	80.6	(10.9)	68.5	(11.2)	67.5	(8.1)
Total recovered	89.8	(10.8)	85.1	(16.1)	81.0	(10.1)
	(% of absorbed)					
Extract	94.2	(1.3)	93.6	(1.7)	95.2	(0.6)

[a] Plants were harvested 5 days after application of ^{14}C-chlorsulfuron to the leaves.
[b] Standard errors of means are in parentheses.

4.4.1.2 Chromatography

Chromatograms obtained by reverse-phase separation of extracts of ^{14}C-chlorsulfuron-treated seedlings (Figure 17) indicate that in flax and wheat one major radioactivity peak was eluted by 35% methanol, and minor peaks by 10, 25, 45, and 100% methanol. In stinkweed, most of the activity was accounted for by three peaks eluted by 25, 35, and 45% methanol. Minor peaks were eluted by 10 and 100% methanol. In all three species very little activity was recovered in the 0% methanol fraction. A ^{14}C-chlorsulfuron standard was eluted as a single peak by 45% methanol.

It is likely, therefore, that the 45% methanol peaks in the chromatograms correspond to unmetabolized ^{14}C-chlorsulfuron.

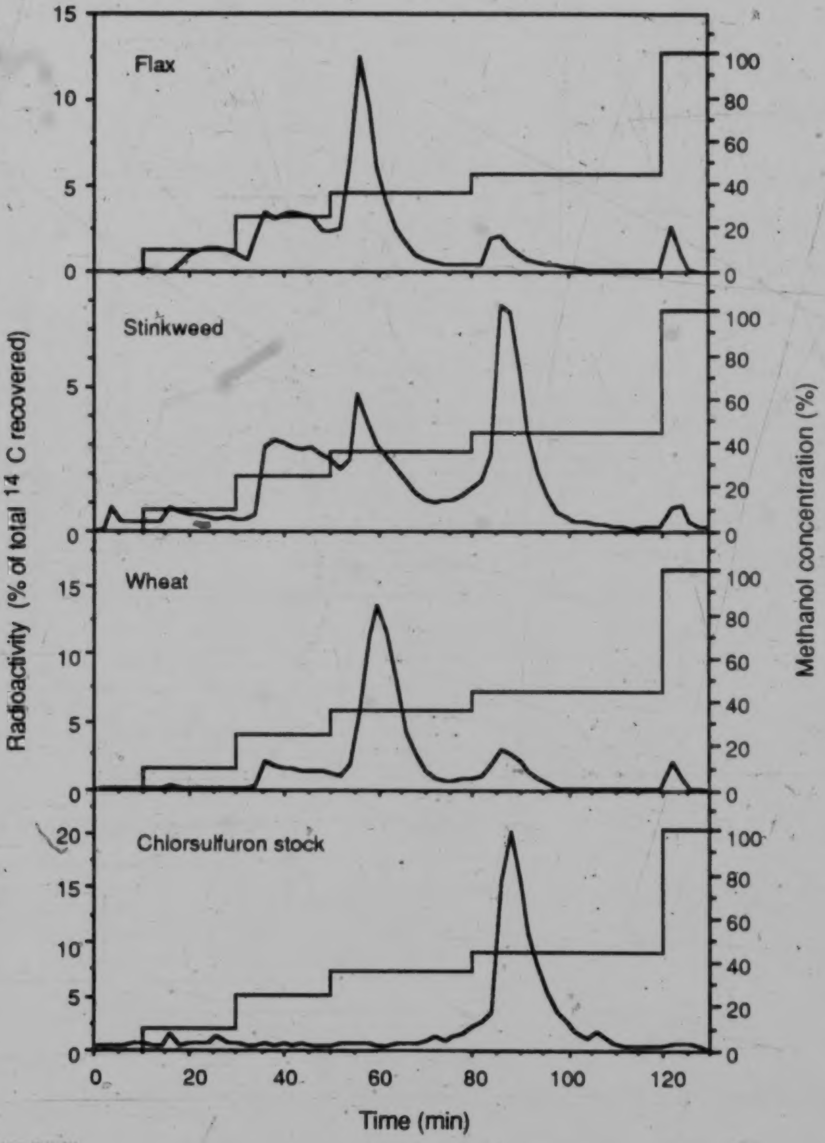


Figure 17. Representative chromatograms obtained by reverse-phase separation of ^{14}C-chlorsulfuron and extracts of ^{14}C-chlorsulfuron-treated flax, stinkweed, and wheat, using a step gradient of aqueous 0.1% (v/v) formic acid and methanol. Flow rate was 0.5 ml/min. The methanol concentration in each step is shown by the straight, stepped line in each chromatogram. Chromatograms were obtained from the extracts of a single plant of each species.

Chlorsulfuron was not degraded during extraction and chromatography. This was demonstrated by adding ^{14}C-chlorsulfuron to untreated flax, stinkweed, or wheat tissue immediately prior to extraction. Upon chromatography of the extracts, 95 to 97% of the activity was recovered as a single peak in the 45% methanol fraction.

The quantitative distribution of ^{14}C-activity among the fractions of the extracts of flax plants, harvested 5 days after treatment, indicates that most of the activity (62%) was recovered in the 35% methanol fraction. (Table 8). The chlorsulfuron-containing fraction (45% methanol) accounted for 12%, and the remaining fractions for 26%. These data confirm the results of Hutchinson *et al.* (1984) who demonstrated that chlorsulfuron is metabolized by flax. In their case about 9% of the recovered activity was chlorsulfuron, 35% was recovered as the methyl-hydroxylated derivative of chlorsulfuron, and 14% as the corresponding *O*-glycoside. They did not account for the remaining 42%.

Table 8. Distribution of ^{14}C-activity among methanol fractions of the extracts of flax, stinkweed, and wheat treated with ^{14}C-chlorsulfuron[a,b].

	^{14}C-distribution					
Methanol fraction	Flax		Stinkweed		Wheat	
(%)	(% of total recovered)					
0	1.4	(0.4)	1.8	(0.5)	2.3	(0.5)
10	6.6	(1.2)	4.3	(1.0)	2.5	(0.3)
25	14.0	(2.8)	16.0	(2.6)	12.8	(1.0)
35	61.6	(3.9)	32.1	(2.8)	74.5	(1.4)
45	12.2	(1.7)	44.5	(4.4)	6.1	(0.7)
100	4.3	(0.8)	1.3	(0.3)	1.7	(0.2)

[a] Plants were harvested 5 days after application of ^{14}C-chlorsulfuron to the leaves.
[b] Standard errors of means are in parentheses.

In stinkweed, which is susceptible to chlorsulfuron, 5 days after treatment approximately 45% of the extracted ^{14}C-activity was eluted by 45% methanol (Table 8). Most of the remaining 55% was in the 25 and 35% methanol fraction.

In wheat, 5 days after treatment, 6% of the recovered ^{14}C-activity was in the 45% methanol fraction, 74% in the 35% methanol fraction, and 20% was distributed among the four remaining fractions. These results compare favourably with those of Sweetser *et al.* (1982), who reported that 24 h after treatment, 6% of the recovered ^{14}C-activity was unmetabolized herbicide, 65% was the *O*-glycoside of chlorsulfuron, and the remaining 29% was accounted for by unidentified products.

4.4.1.3 Identification of the fractions

When the 35% methanol fraction of the wheat extract was incubated with ß-glucosidase and subsequently re-chromatographed, most of the ^{14}C-activity (84%) was eluted with 45% methanol. This shift in elution profile did not occur when the 35% methanol fraction of the wheat extract was incubated with 0.1 M sodium acetate. This suggests that the 35% methanol fraction contains the phenyl glycoside proposed by Sweetser *et al.* (1982), and that the 45% methanol fraction contains the phenyl ring-hydroxylated product. The latter is formed upon cleavage of a glycoside moiety from the phenyl glycoside metabolite. It appears that the phenyl ring-hydroxylated product cannot be separated from chlorsulfuron by the procedure described here. Most likely the peak in the 45% methanol fraction in the chromatogram of the wheat extract shown in Figure 17 represents intact chlorsulfuron, because the hydroxylated intermediate in the metabolism pathway in wheat is only a short-lived product that probably is converted rapidly to the corresponding glycoside conjugate (Sweetser *et al.*, 1982). The elution profiles of the other fractions from the wheat extracts were not changed by incubation with ß-glucosidase.

None of the fractions of flax and stinkweed extracts that were incubated with ß-glucosidase yielded elution profiles that were different from those of the original fractions. This suggests that either no glycoside metabolites are formed in these two species or, if one is formed, it cannot be hydrolyzed by ß-glucosidase or the methyl-hydroxylated product is eluted at the same concentration as the glycoside. This observation is in contrast to the conclusion of Hutchinson *et al.* (1984) who suggested the formation of a major conjugate of chlorsulfuron in flax that could be hydrolyzed by ß-glucosidase, and who identified the hydrolysis product as methyl-hydroxylated chlorsulfuron. Hutchinson *et al.* (1984) harvested the plants 1 day after treatment, while in the present experiments the plants were harvested 5 days after treatment. This longer period may have resulted in further metabolism to products that cannot be hydrolyzed by ß-glucosidase to yield a hydroxylated derivative.

When aliquots of the different fractions of stinkweed and wheat extracts, containing variable amounts of chlorsulfuron equivalents, were applied to stinkweed seedlings, only the 45% methanol fractions inhibited stem elongation (Table 9). Stem elongation was used as a measure of biological activity because it was shown that effects on stem elongation occurred more rapidly, and at lower chlorsulfuron doses, than effects on shoot fresh and dry weight (See section 4.1). No effects on stem elongation were observed when corresponding fractions from untreated wheat plants were applied to stinkweed seedlings. In the case of the flax extracts, both the 0% and the 45% methanol fractions inhibited stem elongation of the seedlings. The effects of the 0% methanol fraction are attributed to severe contact injury produced by this fraction for which there is no ready explanation. Except in the case of the 0% methanol fractions, the amount of chlorsulfuron or chlorsulfuron-derived product that was applied to the seedlings was greater than the minimum amount of chlorsulfuron needed to inhibit growth in these seedlings (approximately 14 picomoles). On average, 40 to 60% of the applied ^{14}C-activity in each fraction was absorbed by the stinkweed seedlings. Hence, the 45% methanol fraction appears to be the only fraction that exhibits biological activity, confirming that this fraction contains chlorsulfuron, or a chlorsulfuron metabolite that is biologically active.

Table 9. The effect of methanol fractions of extracts of chlorsulfuron-treated flax, stinkweed, and wheat on the growth of stinkweed seedlings [a].

	Stinkweed seedling height		
Methanol fraction	Flax extract	Stinkweed extract	Wheat extract
(%)	(cm)		
0	9	22	16
10	24	24	25
25	19	27	23
35	26	16	15
45	2	1	2
100	20	19	22
LSD ($P \leq 0.05$)	14	13	12

[a] Data represent plant height measured 13 days after application of aliquots of the methanol fraction to the leaves.

4.4.2 Extent of chlorsulfuron metabolism in stinkweed seedlings

The extent to which chlorsulfuron was metabolized by stinkweed seedlings, 12 or 24 h after application, is reported in Table 10. The amount of chlorsulfuron absorbed is in fair agreement with the results obtained in the absorption experiments reported in section 4.3. Good recovery of ^{14}C-activity was obtained; very little of the applied radioactivity was lost with the pellets. Chromatography of the extracts indicated that most of the ^{14}C-activity, 85 and 90% at 12 and 24 h after application, respectively, was recovered in the 45% methanol fraction. There is no obvious explanation for the apparent decrease in the extent of metabolism between 12 and 24 h after application. Although stinkweed seedlings can metabolize chlorsulfuron to nonphytotoxic compounds (see sections 4.4.1.2 and 4.4.1.3), at 12 and 24 h after application most (on average 88%) had not been metabolized. These results are similar to the ones reported by Sweetser *et al.* (1982). These authors found that in such susceptible species as sugarbeet (*Beta vulgaris* L.), soybean [*Glycine max* (L.) Merr.], and cotton (*Gossypium hirsutum* L.), 24 h after application of ^{14}C-chlorsulfuron, approximately 97, 85, and 75%, respectively, of the radioactivity was recovered as chlorsulfuron.

4.4.3 Discussion

The aqueous extraction procedure outlined in Figure 5 worked very well for chlorsulfuron, a herbicide of relatively low solubility in water (300 ppm at 25 C, pH 7). The final extract was sufficiently clear that it could be used directly for quantification or chromatographic separation. This is one of the major advantages of this procedure over the organic solvent-based extraction procedures previously published for chlorsulfuron (Hageman and Behrens, 1984a; Hutchinson *et al.*, 1984; Sweetser *et al.*, 1984). The chromatographic procedure described separates chlorsulfuron from its metabolites, except from its phenyl ring-hydroxylated product. The procedure can be used readily to assess the amount of ^{14}C-chlorsulfuron detoxification that has occurred in plants, without resorting to methods that involve HPLC. The procedure appears to be particularly well suited for herbicides that, when metabolized by plants, form products that are considerably more polar than the original molecule. Adaptation to other herbicides might require a different step gradient, depending upon the solubility of the herbicide in question.

Table 10. Metabolism of chlorsulfuron in stinkweed seedlings, assessed 12 and 24 h after the application of ^{14}C-chlorsulfuron to a single leaf. The data are based on two runs with three plants per treatment.

Description	^{14}C-distribution 12 h		^{14}C-distribution 24 h	
	(% of applied)			
Leaf wash	87.9	(1.1)	74.0	(4.8)
Pellet I	0.5	(0.4)	0.3	(0.1)
Pellet II	0.2	(0.1)	0.2	(0.0)
Microfuge pellet	0.2	(0.0)	0.2	(0.1)
Extract	6.4	(0.9)	16.7	(3.1)
Total recovered	95.3	(0.7)	91.4	(1.7)
	(% of absorbed)			
Extract	87.5	(3.0)	95.5	(0.7)
Methanol fraction (%)	(% of total extracted)			
0	0.4	(0.2)	0.3	(0.1)
10	2.0	(0.4)	1.6	(0.1)
25	5.6	(3.1)	1.9	(0.2)
35	3.6	(0.7)	4.2	(0.5)
45	85.0	(3.8)	90.0	(0.8)
100	3.4	(1.3)	2.0	(0.6)

Flax and wheat are tolerant to chlorsulfuron due to their ability to metabolize the herbicide to nonphytotoxic products. Although stinkweed is a susceptible species, the results indicate that it also can metabolize chlorsulfuron to nonphytotoxic products. However, the rate of chlorsulfuron metabolism in stinkweed is much lower than in wheat or flax. In stinkweed, seven times as much chlorsulfuron remains in an unaltered, phytotoxic form as in wheat, five days after application. Hence, the selectivity of chlorsulfuron is based more on relative differences in metabolism, rather than on absolute differences among plant species.

4.5 Effect of chlorsulfuron on the allocation of ^{14}C-activity in intact seedlings following exposure to $^{14}CO_2$

These experiments were designed to determine if the rapid effect of chlorsulfuron on the elongation of an emerging leaf coincided with an effect on carbon metabolism in the shoot apical tissue. Intact seedlings were placed in the assimilation chamber (Figure 8) in such a manner that the herbicide-treated leaf (third true leaf) was in the inner chamber and the remaining part of the shoot in the outer chamber. Only the shoots in the outer chamber were exposed to $^{14}CO_2$. The leaves that were treated with chlorsulfuron were purposely not exposed to $^{14}CO_2$, in order to exclude any possible localized effect of the herbicide on carbon metabolism.

4.5.1 Recovery and allocation of ^{14}C-activity in fractions extracted from nontreated shoot tissue

Chlorsulfuron did not have an effect on the total $^{14}CO_2$-fixation as measured by the ^{14}C-activity that could be recovered in the soluble fractions extracted from the non-treated shoot tissue (Tables 11 and 12). Except in the case of the amino acid fraction extracted from the shoot apical tissue (Table 12), there was no effect of chlorsulfuron on the allocation of ^{14}C-activity among the various fractions and on the quantities of sugars and amino acids in the fractions. In the amino acid fraction

Table 11. **Effect of chlorsulfuron on recovery and allocation of ^{14}C-activity in the various fractions extracted from non-treated shoots (minus apical tissue and herbicide-treated leaf) following exposure (30 min labeling period, 90 min chase period) to $^{14}CO_2$. The data (means with standard errors in parentheses) are based on the results of two runs with 3 seedlings per herbicide treatment.**

Parameter	Chlorsulfuron		Effect[a]	Chlorsulfuron		Effect[a]
Time[b]	Blank	1 µg		Blank	1 µg	
Total recovered						
(h)	---------(dpm/mg)	-------	(%)			
3	2975 (333)	3289 (325)	111			
6	3147 (163)	2755 (188)	88			
9	3390 (175)	3115 (223)	92			
12	2337 (304)	2466 (528)	102			
24	2617 (231)	2443 (213)	94			
Sugars						
(h)	-------(nmoles/mg)	------	(%)	-------(dpm/nmole)	-----	(%)
3	17.0 (4.0)	17.4 (4.3)	108	140.3 (33.8)	164.8 (44.1)	118
6	34.7 (3.0)	30.6 (2.1)	90	69.2 (3.8)	64.6 (3.0)	94
9	43.4 (1.7)	39.1 (2.5)	91	62.5 (3.6)	63.5 (2.0)	102
12	38.7 (4.3)	42.2 (10.0)	109	48.5 (2.9)	48.5 (0.8)	99
24	67.3 (5.2)	62.5 (7.6)	93	32.7 (2.4)	33.6 (1.9)	103
Amino acids						
(h)	-------(nmoles/mg)	------	(%)	-------(dpm/nmole)	-----	(%)
3	13.6 (2.2)	15.4 (0.5)	111	25.5 (2.9)	22.6 (1.9)	90
6	16.7 (1.6)	16.3 (0.8)	99	17.4 (0.8)	16.2 (1.3)	93
9	18.2 (2.0)	16.7 (1.2)	91	16.2 (1.2)	14.9 (2.1)	90
12	15.0 (1.0)	15.3 (1.4)	108	13.7 (1.3)	10.6 (0.7)	78
24	16.3 (1.2)	15.3 (1.4)	95	10.8 (0.7)	10.1 (1.1)	94
Acid-1 fraction						
(h)	---------(dpm/mg)	-------	(%)			
3	690 (26)	660 (66)	95			
6	465 (20)	462 (29)	99			
9	363 (34)	352 (19)	99			
12	208 (28)	226 (17)	107			
24	235 (14)	214 (18)	92			
Acid-2 fraction						
(h)	---------(dpm/mg)	-------	(%)			
3	52 (9)	47 (4)	92			
6	31 (3)	36 (4)	118			
9	33 (4)	34 (4)	106			
12	24 (4)	20 (1)	86			
24	19 (4)	16 (2)	92			

---

[a] Expresses the chlorsulfuron-induced effect in terms of a percentage of the control seedlings ('Blank'). The values listed are means of percentage values for the two separate runs.

[b] Refers to the time interval between application of the herbicide and exposure to $^{14}CO_2$.

Table 12. Effect of chlorsulfuron on recovery and allocation of ^{14}C-activity in the various fractions extracted from shoot apical tissue following exposure (30 min labeling period, 90 min chase period) of the shoot to $^{14}CO_2$. The data (means with standard errors in parentheses) are based on the results of two runs with 3 seedlings per herbicide treatment.

Parameter	Chlorsulfuron		Effect[a]	Chlorsulfuron		Effect[a]
Time[b]	Blank	1 µg		Blank	1 µg	
Total recovered						
(h)	---------(dpm/mg)	-------	(%)			
3	3345 (521)	3552 (487)	105			
6	2751 (407)	4201 (910)	170			
9	4093 (310)	4166 (292)	103			
12	3361 (321)	2621 (203)	78			
24	2991 (213)	2763 (394)	92			
Sugars						
(h)	-------(nmoles/mg)	------	(%)	-------(dpm/nmole)	-----	(%)
3	31.8 (2.8)	40.9 (9.8)	128	40.7 (8.9)	43.9 (8.3)	108
6	29.0 (4.4)	36.4 (5.6)	126	40.3 (7.3)	53.9 (7.2)	140
9	48.1 (5.1)	45.9 (6.7)	97	53.2 (7.0)	50.1 (5.3)	96
12	47.1 (4.5)	42.1 (4.9)	90	42.4 (6.9)	37.7 (5.6)	89
24	51.7 (4.0)	58.0 (4.3)	113	37.3 (2.2)	34.2 (5.1)	92
Amino acids						
(h)	-------(nmoles/mg)	------	(%)	-------(dpm/nmole)	-----	(%)
3	118.5 (16.2)	97.7 (15.3)	86	13.5 (2.3)	16.9 (4.3)	130
6	199.6 (37.9)	307.6 (78.1)	150	6.8 (1.0)	5.9 (1.2	84
9	146.4 (53.9)	191.2 (30.1)	149	10.4 (2.1)	6.8 (1.2)	66
12	79.3 (7.4)	130.9 (14.6)	171	9.7 (0.7)	4.8 (0.5)	53
24	115.4 (28.1)	115.6 (17.4)	109	8.3 (2.4)	4.9 (0.8)	70
Acid-1 fraction						
(h)	---------(dpm/mg)	-------	(%)			
3	544 (68)	592 (79)	108			
6	297 (110)	734 (214)	975			
9	452 (81)	649 (112)	141			
12	490 (66)	366 (32)	75			
24	324 (30)	244 (44)	76			
Acid-2 fraction						
(h)	---------(dpm/mg)	-------	(%)			
3	97 (29)	129 (32)	142			
6	69 (13)	110 (35)	199			
9	80 (18)	134 (52)	164			
12	176 (89)	106 (33)	72			
24	61 (9)	60 (14)	97			

---

[a] Expresses the chlorsulfuron-induced effect in terms of a percentage of the control seedlings ('Blank'). The values listed are means of percentage values for the two separate runs.

[b] Refers to the time interval between application of the herbicide and exposure to $^{14}CO_2$.

extracted from the shoot apical tissue there was a decline in the amount of ^{14}C-activity incorporated as the time interval between the application of chlorsulfuron and the exposure to $^{14}CO_2$ increased. In the non-treated shoot tissue there was an increase in the amount of sugars as the time interval between the chlorsulfuron treatment and the exposure to $^{14}CO_2$ increased. However, this increase occurred in both the blank and the chlorsulfuron-treated seedlings and must be attributed to factors other than the herbicide treatment.

From the data in Table 11 it can be calculated that in the non-apical shoot tissue, most (77.5%) of the ^{14}C-activity was recovered in the sugar fraction; 8.5%, 13.0%, and 1.0% was recovered in the amino acid, the acid-1, and the acid-2 fractions, respectively. In the shoot apical tissue (Table 12), 50.8% of the recovered ^{14}C-activity was in the sugar fraction, while in the amino acid, the acid-1, and the acid-2 fractions, the allocation was 33.6%, 12.8%, and 2.8%, respectively.

4.5.2 Translocation of ^{14}C-activity into chlorsulfuron-treated Leaves

When the non-treated shoots in the outer part of the assimilation chamber were exposed to $^{14}CO_2$, very little ^{14}C-activity was translocated to the leaves situated in the inner part of the chamber. On average only about 0.14% of the total ^{14}C-activity recovered from the shoot was recovered from the leaves in the inner chamber. Treating the leaves in the inside chamber with 1 μg of chlorsulfuron up to 24 hours prior to exposing the shoots in the outside chamber to $^{14}CO_2$ had no effect on the translocation of ^{14}C-activity into the treated leaves in the inner chamber.

4.5.3 Discussion

The reduction in the accumulation of ^{14}C-activity in the amino acid fraction extracted from the shoot apical tissue, without a concomitant reduction in the total amount of amino acids in that fraction, suggests an effect of chlorsulfuron on amino acid metabolism. On the basis of the site of action of chlorsulfuron (Ray, 1984a), it is expected that the reduction in ^{14}C-activity in the amino acid fraction was due to the inhibition of the synthesis of the branched-chain amino acids. Attempts were made to quantify the individual amino acids in the amino acid fractions, and to determine the total amount of ^{14}C-activity incorporated into each one of them. However, due to the low specific activity of the individual amino acids, the minimum size of the sample to be separated to detect the ^{14}C-activity in each amino acid exceeded the capacity of the chromatography column. It was expected that one of the first effects of chlorsulfuron would have been a reduction in the incorporation of ^{14}C-activity into L-valine, L-leucine, and L-isoleucine.

4.6 Effect of chlorsulfuron on assimilate translocation

Both intact seedlings and excised leaves were used to study the effect of chlorsulfuron on the transport of assimilates out of the treated leaf.

4.6.1 Intact seedlings

One microgram of chlorsulfuron, applied to single leaves of intact stinkweed seedlings, reduced the translocation of ^{14}C-labelled assimilates out of these leaves, if they were exposed to $^{14}CO_2$ 12 or 24 h after the application of the herbicide (Figure 18). No such reduction occurred when $^{14}CO_2$ was applied 6 h after the herbicide treatment. Application of 1 µg of chlorsulfuron to one leaf of an intact seedling had no effect on the translocation of assimilates out of the adjacent leaf when it was exposed to $^{14}CO_2$ 6 or 12 h after the herbicide application. In this instance, a period of 24 h between chlorsulfuron application and exposure to $^{14}CO_2$ was required in order to observe a reduction in assimilate translocation. Chlorsulfuron had no effect on the total amount of $^{14}CO_2$ assimilated by the leaves.

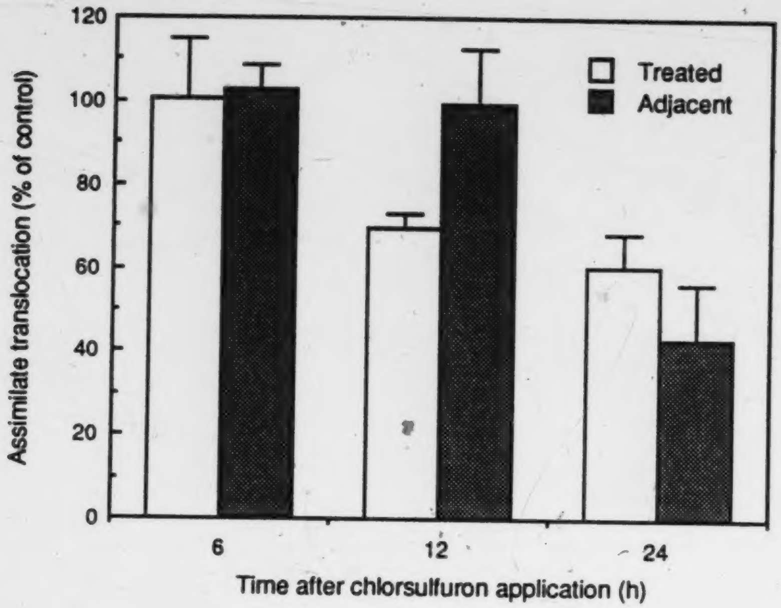


Figure 18. Effect of 1 µg of chlorsulfuron, applied to a single leaf of intact stinkweed seedlings, on the translocation of ^{14}C-assimilates out of the treated leaf, or the leaf adjacent to the treated leaf. Leaves were exposed to $^{14}CO_2$ (30 min pulse, 90 min chase period) 6, 12, or 24 h after herbicide application. The data, means and standard errors, are expressed as a percentage of the control, and are based on the results of three runs with three seedlings per treatment.

4.6.2 Excised leaves

Excised leaves, exuding through the cut end of the petiole into an EDTA-containing buffer solution, are a simple system in which the effect of a herbicide treatment on the assimilate source tissue can be studied (Groussol *et al.*, 1986; King and Zeevaart, 1974). One of the keys to the succesful functioning of this system is the concentration of the chelating agent EDTA in the bathing solution (Groussol *et al.*, 1986; Fellows and Zeevaart, 1983). EDTA forms stable complexes with divalent cations; it reduces the concentration of freely available Ca^{2+} ions in the bathing solution. Free Ca^{2+} ions participate in the reactions that seal cut phloem tissue; EDTA prevents this sealing, caused by the formation of the polysaccharide callose, and enables excised leaves to exude.

4.6.2.1 Optimum EDTA concentration

The optimum EDTA concentration for the exudation of sugars by excised stinkweed leaves ranged from 0.5 to 5 mM, depending upon the length of the exudation period (Figure 19). At all EDTA concentrations the leaves remained turgid for the duration of the 8-h exudation period. At the end of this period, only the petioles of the leaves in the bathing solution containing 5, 10, and 20 mM EDTA had become translucent. This is suggested to be a sign of EDTA phytotoxicity (Groussol *et al.*, 1986).

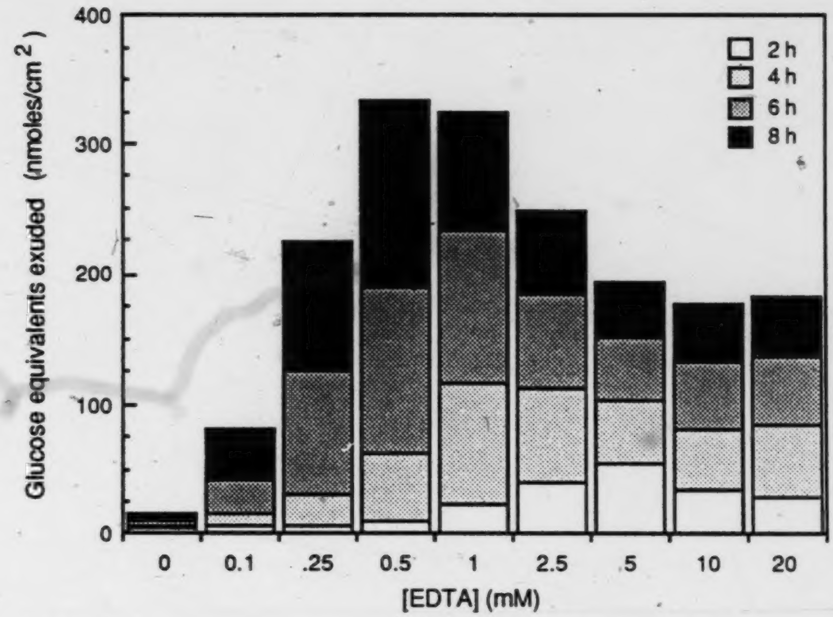


Figure 19. Effect of increasing concentration of EDTA in the bathing solution (5 mM phosphate buffer; pH 6.0) on the exudation of anthrone-positive compounds by the petioles of excised leaves of stinkweed seedlings. The data (means) are expressed as the cumulative amount of glucose equivalents exuded per cm^2 of leaf area at 2, 4, 6, and 8 h after excision, and are the results of one run with eight leaves per EDTA concentration.

4.6.2.2 Exudation following $^{14}CO_2$-application.

When stinkweed seedling leaves that had been treated with 1 µg of chlorsulfuron were excised and exposed to $^{14}CO_2$, 12 or 24 h after herbicide application, an effect on the exudation profiles of both sugars and ^{14}C-activity was observed (Figure 20). For both time intervals, less ^{14}C-activity and less sugar was exuded by the chlorsulfuron-treated leaves during the subsequent 13-h exudation period. With a 6-h time interval between the application of chlorsulfuron and exposure to $^{14}CO_2$, only a reduction in the total amount of sugar exuded by the leaves could be observed. There was no significant effect on the amount of ^{14}C-activity exuded.

The reduction in the exudation of sugars and ^{14}C-activity by the chlorsulfuron-treated leaves could be prevented if, 6 to 8 h prior to herbicide application, the roots of the seedlings were placed in nutrient solution containing 1 mM of each of the amino acids L-valine, L-leucine, and L-isoleucine (Figure 21). These amino acids completely alleviated the effect of the herbicide.

The reduction in sugar and ^{14}C-activity exudation also occurred when, 24 h after the seedlings had been sprayed with chlorsulfuron at a rate of 11.25 g/ha, the third true leaf was excised and exposed to $^{14}CO_2$ (Figure 22).

Exudation of ^{14}C-activity and of sugars by the leaf adjacent to the chlorsulfuron-treated leaf was not affected by a chlorsulfuron treatment applied 24 h prior to excision and exposure to $^{14}CO_2$ (Figure 23).

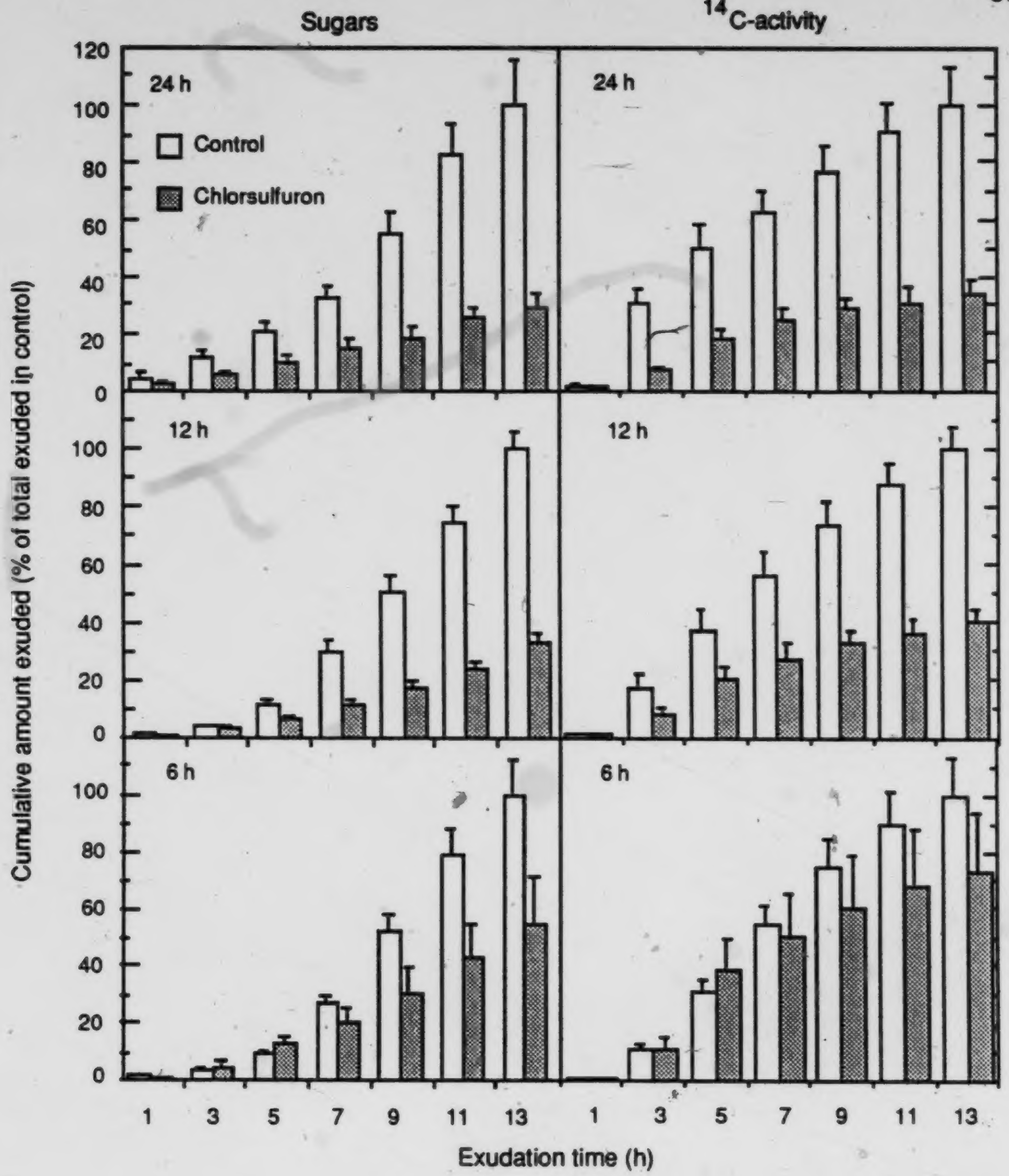


Figure 20. Exudation profiles of single, excised stinkweed leaves, following application of blank or 1 μg of chlorsulfuron to the leaves, 6, 12, or 24 h prior to excision and exposure to $^{14}CO_2$ (30 min pulse). The data, means and standard errors, are expressed as percentages of the cumulative amount of ^{14}C-activity or sugars exuded by the control leaves during the 13-h exudation period, and are the results of two runs with three leaves per herbicide dose.

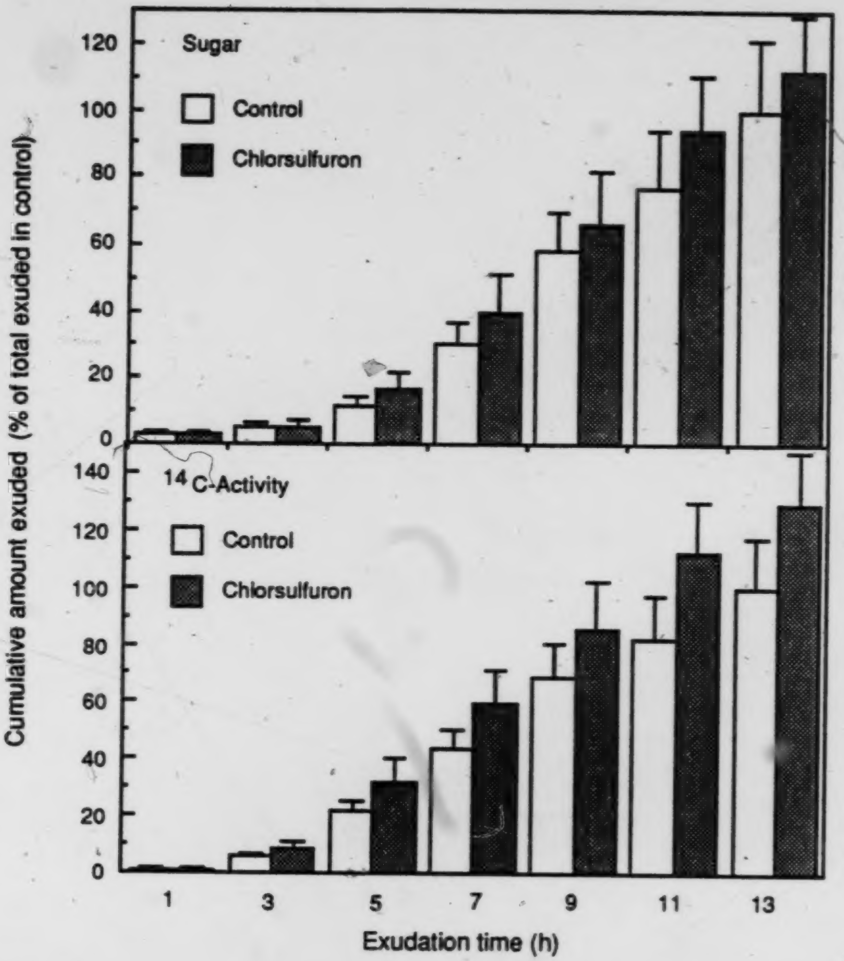


Figure 21. Exudation profiles of single, excised stinkweed leaves, following application of blank or 1 µg of chlorsulfuron to the leaves 24 h prior to excision and exposure to $^{14}CO_2$ (30 min pulse). Six to eight hours prior to herbicide application the seedlings were placed with their roots in half-strength Hoagland's solution containing 1 mM of each of the amino acids L-valine, L-leucine, and L-isoleucine. The data, means and standard errors, are expressed as percentages of the cumulative amount of ^{14}C-activity or sugars exuded by the control leaves during the 13-h exudation period, and are the results of two runs with three leaves per herbicide dose.

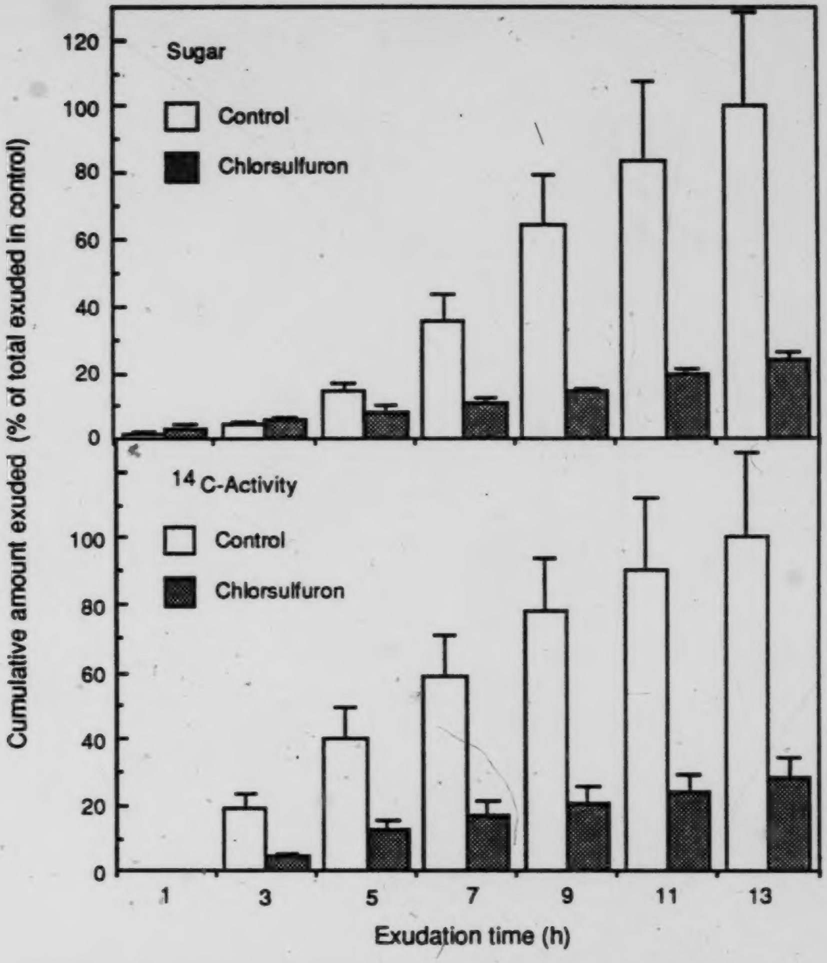


Figure 22. Exudation profiles of single, excised stinkweed leaves, following a spray application of 0 or 11.25 g /ha of chlorsulfuron, 24 h prior to excision and exposure to $^{14}CO_2$. The data, means and standard errors, are expressed as percentages of the cumulative amount of ^{14}C-activity or sugars exuded by the control leaves during the 13-h exudation period, and are the results of two runs with three leaves per herbicide dose.

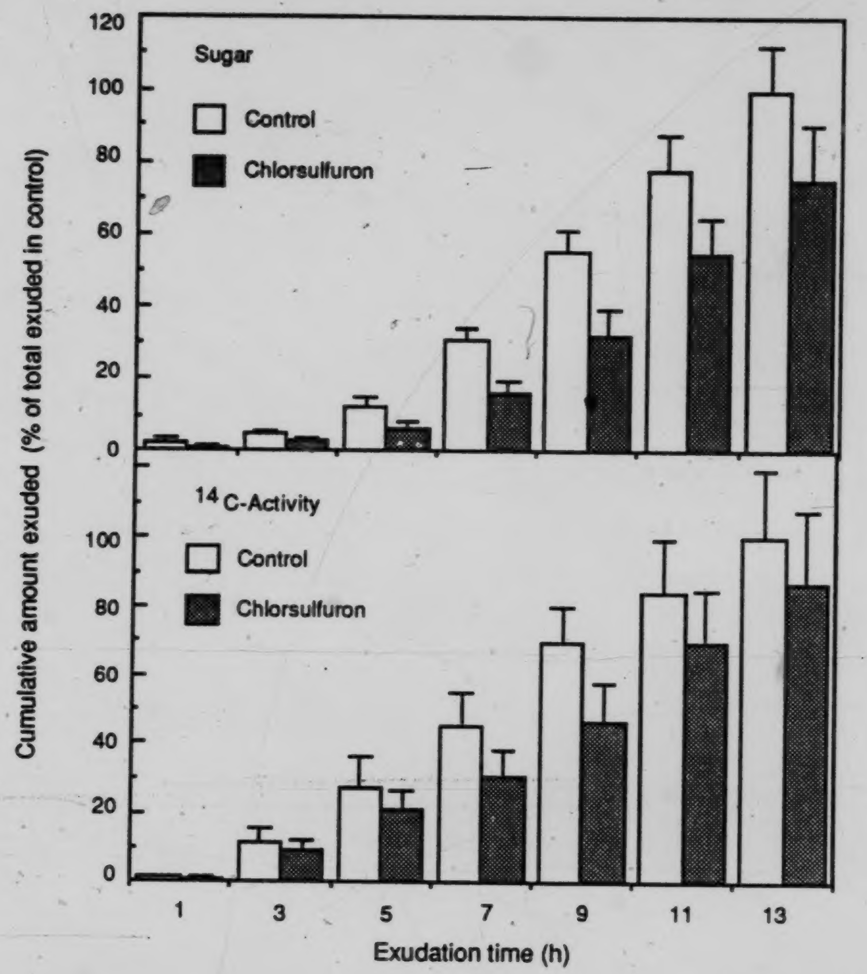


Figure 23. Exudation profiles of single, excised stinkweed leaves, following application, 24 h prior to excision and exposure to $^{14}CO_2$, of blank or 1 μg of chlorsulfuron to the leaf adjacent to the excised leaf. The data, means and standard errors, are expressed as percentages of the cumulative amount of ^{14}C-activity or sugars exuded by the control leaves during the 13-h exudation period, and are the results of two runs with three leaves per herbicide dose.

4.6.2.3 Exudation following ^{3}H-sucrose and ^{14}C-sucrose application

When, 12 h after the application of 1 μg of chlorsulfuron, leaves of stinkweed seedlings were excised and ^{3}H-sucrose and ^{14}C-sucrose were applied, no effect on the ^{3}H-activity and ^{14}C-activity exudation profiles was observed (Figure 24). Neither was there an effect on the sugar exudation profiles. Leaf excision and application of ^{3}H-sucrose and ^{14}C-sucrose 24 h after the application of chlorsulfuron resulted in a decrease in the exudation of ^{14}C-activity and of sugars (Figure 25). However, there was no effect on the exudation of ^{3}H-activity. These results suggest that chlorsulfuron has a localized effect, since the ^{3}H-sucrose was applied to the non-treated areas of the leaves and the ^{14}C-sucrose was applied to the herbicide-treated areas of the leaves. The decrease in exudation occurred only when sucrose was applied to the herbicide-treated area of the leaf. Chlorsulfuron does not appear to affect the untreated area of the leaf. Sucrose applied to that part of the leaf exuded at the same rate as that of leaves not treated with herbicide. The effect of chlorsulfuron (on the treated half of the leaf) was prevented if the seedlings were placed with their roots in nutrient solution containing 1 mM of L-valine, L-leucine, and L-isoleucine, 6 to 8 h prior to the chlorsulfuron treatment.

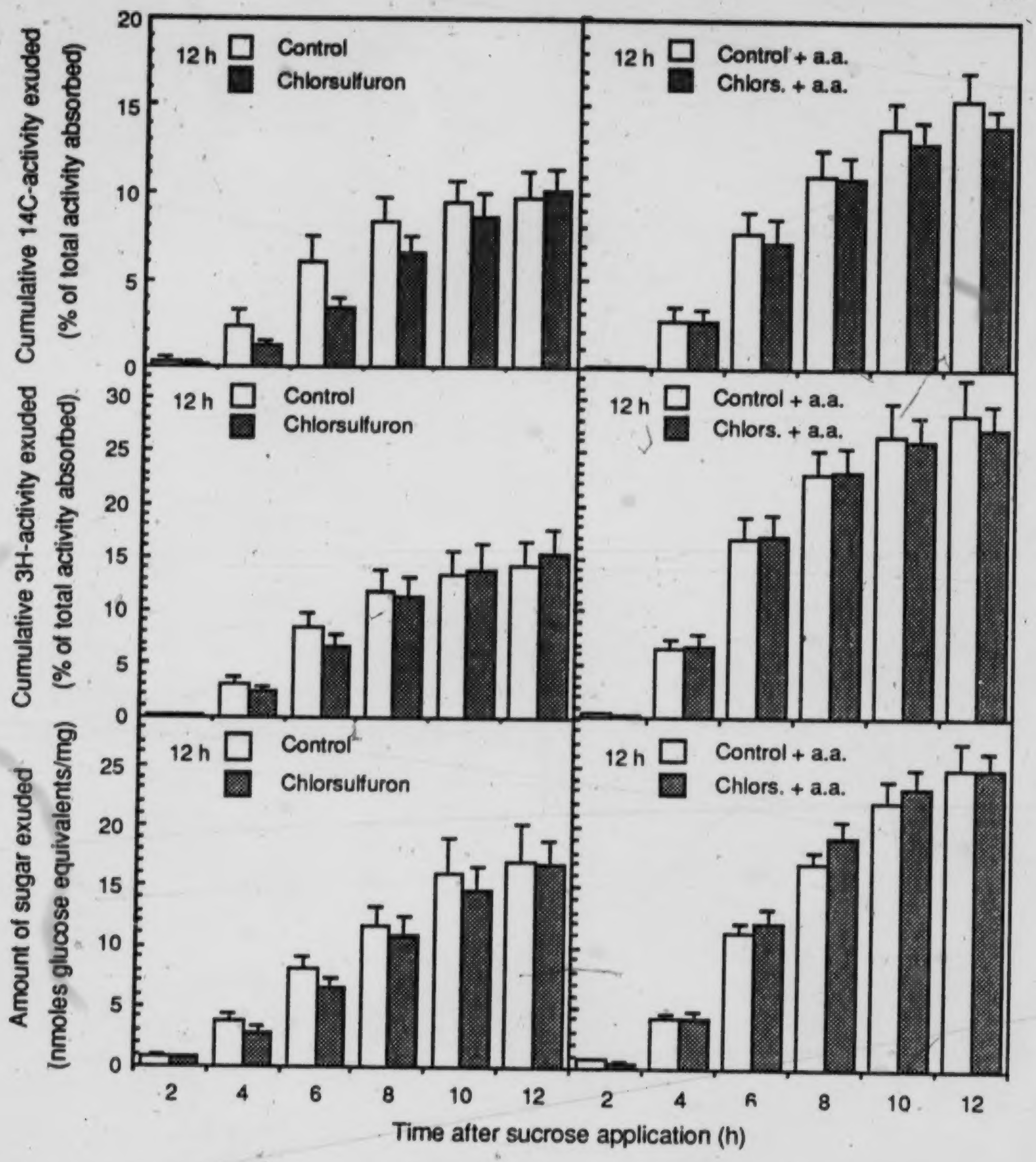


Figure 24. Exudation profiles of single, excised stinkweed leaves, following application of 0 or 1 μg of chlorsulfuron to the leaves, 12 h prior to treatment with ^{3}H- and ^{14}C-sucrose. Six to eight hours prior to herbicide application the seedlings were placed with their roots in half-strength Hoaglands' solution or in half-strength Hoaglands' solution containing 1 mM of each of the amino acids L-valine, L-leucine, and L-isoleucine. ^{14}C-sucrose was applied to the area of the leaf that had been treated with herbicide; ^{3}H-sucrose was applied to the area that had not been treated with herbicide. The data, means and standard errors, are expressed as nanomoles of glucose equivalents exuded per mg of tissue, or as the cumulative amount of ^{3}H- or ^{14}C-activity exuded as a percentage of the total activity absorbed; they are the results of one run with six leaves per herbicide dose.

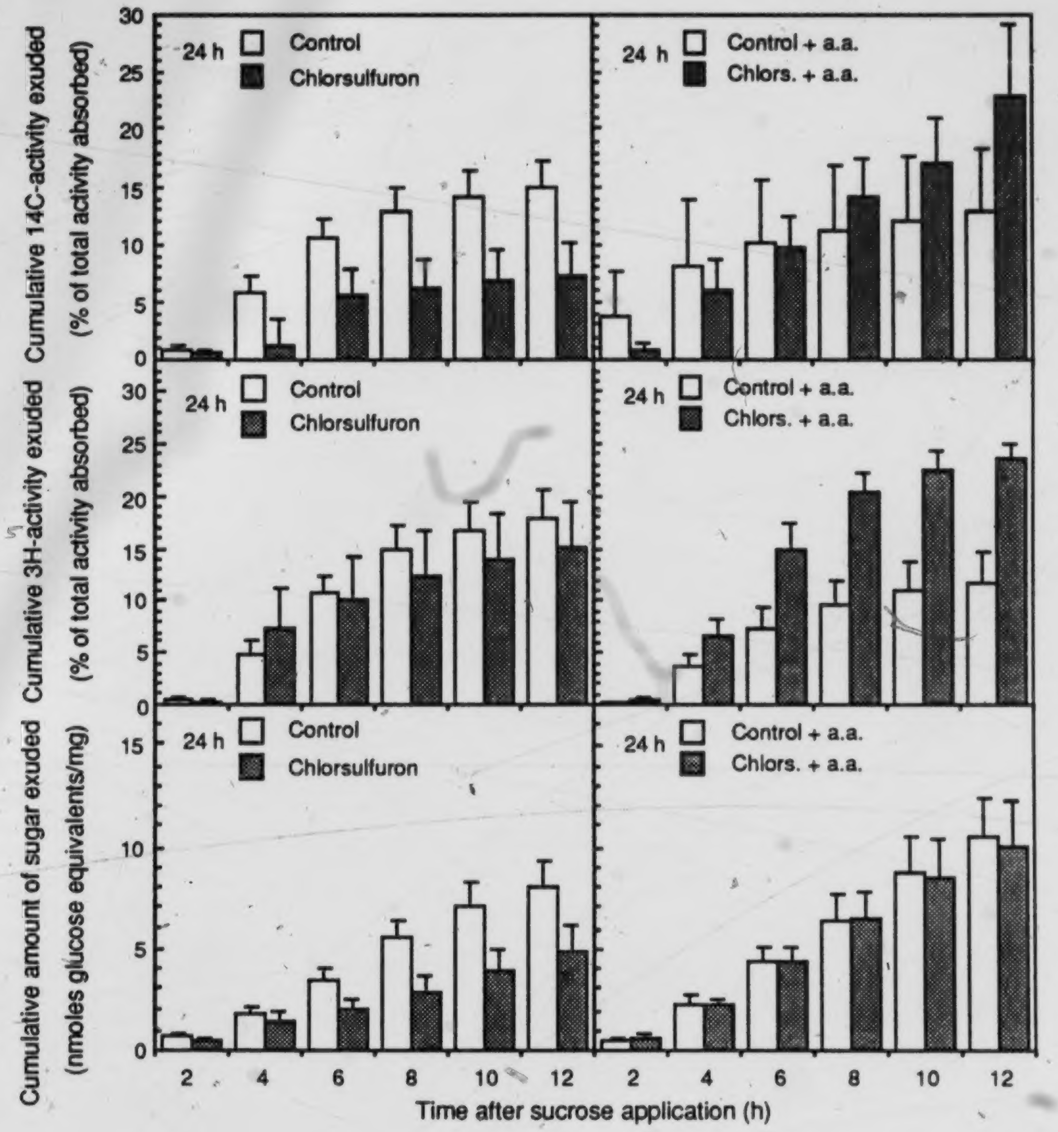


Figure 25. Exudation profiles of single, excised stinkweed leaves, following application of 0 or 1 µg of chlorsulfuron to the leaves, 24 h prior to treatment with ^{3}H- and ^{14}C-sucrose. See Figure 24 for other experimental details.

4.6.3 Discussion

The absence of an effect of chlorsulfuron on the total amount of $^{14}CO_2$ asssimilated by the treated leaves suggests that in the time period studied (maximum of 24 h) the herbicide had no measurable effect on photosynthesis. This confirms earlier reports (Hatzios and Howe, 1982).

The reduction in the export of ^{14}C-activity out of the chlorsulfuron-treated leaves of intact seedlings indicates that the herbicide had an effect on assimilate translocation. This was also demonstrated by the experiments with excised leaves, where the herbicide caused a reduction in the exudation of both ^{14}C-activity and anthrone-positive sugars. The ability of the branched-chain amino acids to overcome this reduction in exudation strongly suggests that the observed reduction was the result of the mechanism of action of chlorsulfuron.

The localized effect of chlorsulfuron on the assimilate transport system, suggested by the results of the sucrose experiments, is consistent with the findings[46] that chlorsulfuron does not spread throughout a leaf readily, if at all, following a spot application. Presumably, if sufficient herbicide had moved from the treated areas of the leaves to the non-treated ones, a reduction in the exudation of ^{3}H-activity would have been observed.

The agreement between the results obtained with intact seedlings and those with excised leaves occurred despite large differences in the amount of ^{14}C-activity translocated or exuded in the control seedlings or excised leaves. In intact seedlings 16% of the total ^{14}C-activity assimilated was translocated out of the third true leaf, 2 h after it had been exposed to $^{14}CO_2$. Excised leaves exuded only 4.6% of the total amount of ^{14}C-activity assimilated during the 13-h period following exposure to $^{14}CO_2$.

---

[46] M.D. Devine, personal communication.

Excised bean leaves, by comparison, in 12 h exuded 15% of the total ^{14}C-activity assimilated (Fellows and Zeevaart, 1983). In addition to the species difference, these authors used a much higher concentration of EDTA (20 mM) than excised stinkweed leaves could tolerate.

A discussion of the relationship between the concentration of chlorsulfuron in the tissue and the effect on assimilate transport or exudation is presented in section 4.9.

4.7 Effect on incorporation of ^{14}C-activity into various fractions following exposure to $^{14}CO_2$

Chlorsulfuron had no effect on the total amount of ^{14}C-activity assimilated by the excised leaves of stinkweed seedlings, following exposure to $^{14}CO_2$ 24 h after herbicide application (Table 13). The total amount of sugar and the total amount of ^{14}C-activity exuded by chlorsulfuron-treated leaves was reduced. These results are similar to those reported in section 4.6.2.2.

Analysis of the fractions extracted from the excised leaves demonstrated an increase of 50 and 70%, respectively, in the total amount of soluble sugars and amino acids (Table 13). Only in the case of the sugar fraction was there a concomitant increase in the amount of ^{14}C-activity recovered in that fraction. The amount of ^{14}C-activity recovered in the amino acid fraction was not affected by chlorsulfuron.

Chlorsulfuron reduced the total amount of ^{14}C-activity recovered in both the organic acid and the lipid fraction. The chlorsulfuron-induced reduction in the total amount of ^{14}C-activity recovered in the lipid fraction was due mainly to a reduction in the amount incorporated in the galactolipids.

Table 13. Effect of chlorsulfuron on assimilation and exudation by excised leaves of stinkweed seedlings, and the allocation of ^{14}C-activity in various fractions extracted from these leaves following exposure to $^{14}CO_2$. The leaves were excised and exposed to $^{14}CO_2$ (30 min pulse; 330 min chase period) 24 h after the application of blank or 1 µg of chlorsulfuron. The data are based on the results of two runs with three leaves per treatment.

Parameter *Fraction*	Units[a]	Chlorsulfuron Blank	 1 µg	Herbicide effect as percentage of control	
Assimilation					
^{14}C-activity assimilated	dpm/mg	31,759	33,943	104	N.S.
Exudation					
Total ^{14}C-activity	dpm/mg	1,477	763	50	**
Total sugars	nmole/mg	2.02	1.06	53	**
Leaf extracts					
Sugars					
Total sugar	nmole/mg	34.0	49.4	150	*
Total ^{14}C-activity	dpm/mg	12,891	21,353	163	*
Specific activity	dpm/nmole	381	436	108	N.S.
Amino acids					
Total amino acids	nmole/mg	16.8	28.6	170	**
Total ^{14}C-activity	dpm/mg	2,245	2,315	105	N.S.
Specific activity	dpm/nmole	138	88	64	**
Organic acids					
Total ^{14}C-activity	dpm/mg	6,007	3,453	58	**
Lipids					
Total ^{14}C-activity	dpm/mg	1,249	951	75	*
Neutral lipids	dpm/mg	304	229	77	N.S.
Galactolipids	dpm/mg	367	237	64	*
Phospholipids	dpm/mg	578	485	88	N.S.
Protein					
Total amino acids	nmole/mg	308	257	74	N.S.
Total ^{14}C-activity	dpm/mg	7,596	5,488	71	*
Specific activity	dpm/nmole	34	31	90	N.S.

---

[a] Weights (mg) refer to tissue fresh weights.

[b] N.S. not significant; * significant at $P \le 0.05$; ** significant at $P \le 0.01$.

Analysis of the individual sugars in the sugar fractions showed that one of the effects of chlorsulfuron was an increase in the amounts of sucrose in the treated leaves (Table 14). No increase in the amounts of glucose and fructose could be demonstrated. In the case of fructose was there a small increase in the specific activity.

In order to analyze the protein in the pellets left over after the extraction of the sugars, amino acids, organic acids, and lipids from the leaf tissue, the pellets were incubated with pronase. This enzyme hydrolyzes the peptide bonds in the proteins, yielding free amino acids. Analysis of these amino acids showed that chlorsulfuron had no effect on the total amount of amino acids derived from the leaf proteins (Table 13). The herbicide did reduce the total amount of ^{14}C-activity incorporated in these amino acids. However, the data were highly variable, and caution must be used in interpreting them.

The increase both in the total amount of sugars in the leaves and the amount of ^{14}C-activity activity incorporated in them, coupled with the decrease in the amount of sugars exuded by these leaves, suggest that chlorsulfuron probably does not have an effect on the synthesis of sugars, but rather that it reduces their export out of the leaves. This is supported by the increase in the concentration of sucrose in the chlorsulfuron-treated leaves.

Table 14. Effect of chlorsulfuron on the amounts of sucrose, glucose, and fructose and their specific activities following exposure of excised stinkweed leaves to $^{14}CO_2$. The leaves were excised and exposed to $^{14}CO_2$ (30 min pulse; 330 min chase period) 24 h after the application of blank or 1 µg of chlorsulfuron. The data are based on the results of two runs with three leaves per treatment.

Sugar *Parameter*	Units[a]	Chlorsulfuron Blank	 1 µg	Herbicide effect as percentage of control	
Sucrose					
Total amount	nmole/mg	1.7	4.2	255	*
Specific activity	dpm/nmole	1,021	734	75	N.S.
Glucose					
Total amount	nmole/mg	11.9	16.0	144	N.S.
Specific activity	dpm/nmole	369	408	102	N.S.
Fructose					
Total amount	nmole/mg	5.7	7.4	140	N.S.
Specific activity	dpm/nmole	557	739	127	**

[a] Weights (mg) refer to tissue fresh weights.

[b] N.S. not significant; * significant at $P \leq 0.05$; ** significant at $P \leq 0.01$.

In the case of the amino acid fraction, it must be stated that both the size of the pools of individual amino acids, or of groups of biosynthetically related amino acids, and the flux of the carbon atoms through them, are under metabolic control. It could be postulated that a reduction in the biosynthesis of one group of amino acids, i.e., the branched-chain amino acids, might not necessarily result in an immediate decrease in the total amount of amino acids in the leaf tissue. On the contrary, a decrease in the synthesis of branched-chain amino acids might first of all result in a decrease in protein synthesis, due to a lack of available branched-chain amino acids. This decrease in protein synthesis might result in an accumulation of nonbranched-chain amino acids. The overall effect could be an increase in the total amount of amino acids in the tissue. In the absence of data on the composition of the amino acid fractions and on the total amount of ^{14}C-activity incorporated in each amino acid, the details of the effect of chlorsulfuron on the amino acid metabolism in stinkweed leaves remain speculative.

Due to lack of data on the total amount of organic acids and lipids extracted from the leaf tissue, interpretation of the observed reduction in the incorporation of ^{14}C-activity in the corresponding fractions is difficult. The reductions might be due to a general decrease in the amount of lipids and organic acids present in the leaf tissue, or they might reflect a decrease in the rate of synthesis of these compounds. On the basis of the data presented in Table 13, no distinction between these two possibilities can be made.

4.8 Effect on incorporation of ^{14}C-activity into soluble proteins following exposure of excised leaves to $^{14}CO_2$

In light of the large variability in both the amount and the specific activity of the amino acids that were enzymatically liberated from the proteins in the pellets remaining after the extraction of the various fractions (Table 13), a series of experiments was conducted to study specifically the effect of chlorsulfuron on the incorporation of ^{14}C-activity into soluble leaf proteins following exposure of excised leaves to $^{14}CO_2$. The results of these experiments indicate that chlorsulfuron has no effect on the total amounts of protein extracted from the excised leaves (Table 15). In addition, the herbicide had no effect on the specific activity of the proteins in these extracts, determined by assaying an aliquot of the protein extracts by means of LSS. However, when the proteins in the extracts were separated by means of SDS-PAGE, and the ^{14}C-activity in the polyacrylamide gels was visualized fluorographically, a reduction in the amount of ^{14}C-activity incorporated in the proteins extracted from leaves that had been treated with chlorsulfuron was observed (Figure 26).

Table 15. Effect of chlorsulfuron on the amount of soluble proteins and on the incorporation of ^{14}C-activity into these proteins following exposure of excised leaves of stinkweed seedlings to $^{14}CO_2$. The leaves were excised and exposed to $^{14}CO_2$ (30 min pulse; 330 min chase period) 24 h after the application of blank or 1 µg of chlorsulfuron. Six to eight hours prior to herbicide application the seedlings were placed with their roots in half-strength Hoaglands' solution or in half-strength Hoaglands' solution containing 1 mM of each of the amino acids L-valine, L-leucine, and L-isoleucine. The data are based on the results of two runs with three leaves per treatment.

Parameter	Seedlings treated with branched-chain amino acids	Units[a]	Chlorsulfuron		Herbicide effect as percentage of control	
			Blank	1 µg		
Amount of protein	no	µg/mg	10.43	11.37	109	N.S.
	yes	µg/mg	12.25	12.93	106	N.S.
Specific activity of protein	no	dpm/µg	271	285	105	N.S.
	yes	dpm/µg	85	103	122	N.S.
Amount of amino acids in protein	no	nmole/µg	23	25	106	N.S.
	yes	nmole/µg	21	20	98	N.S.
Specific activity of amino acids	no	dpm/nmole	2.46	1.40	57	**
	yes	dpm/nmole	0.84	1.01	119	*

[a] Weights (mg) refer to tissue fresh weights.

[b] N.S. not significant; * significant at P ≤ 0.05; ** significant at P ≤ 0.01.

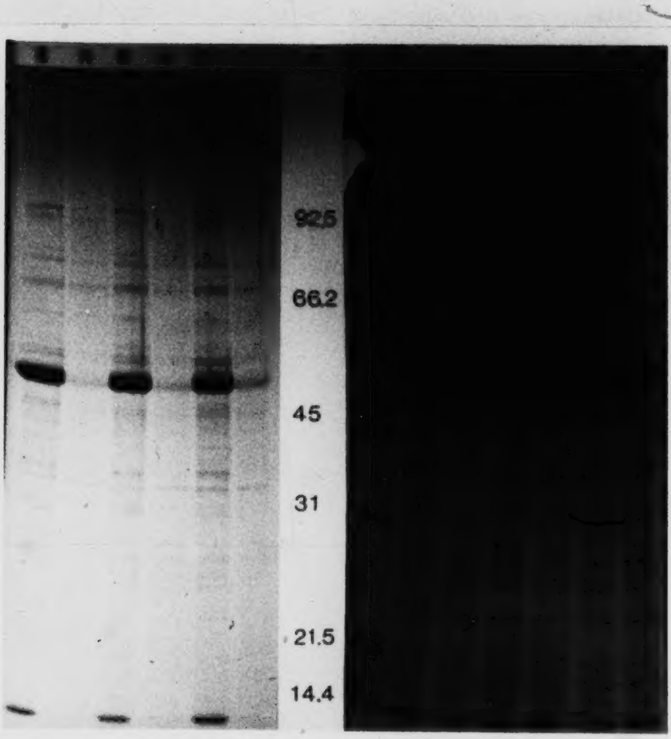


Figure 26. Combined SDS-PAGE (right) and corresponding fluorogram (left) of soluble proteins extracted from excised leaves of stinkweed seedlings exposed to $^{14}CO_2$. The leaves were excised and exposed to $^{14}CO_2$ (30 min pulse; 330 min chase period) 24 h after the application of blank (B) or 1 µg of chlorsulfuron (H). The molecular weight markers are (top to bottom): phosphorylase B (92.5 kD); bovine serum albumin (66.2 kD); ovalbumin (45 kD); carbonic anhydrase (31 kD); soybean trypsin inhibitor (21.5 kD); lysozyme (14.4 kD). A total of 80 µg of protein was loaded in each lane.

This observed reduction in the amount of ^{14}C-activity incorporated into the soluble leaf proteins was quantified by hydrolyzing the proteins in the different extracts with methane sulfonic acid, purifying the amino acids in the hydrolysates by means of cation exchange chromatography, and determining the specific activity of these amino acids. The results support the evidence obtained with the polyacrylamide gels. Chlorsulfuron reduced the incorporation of ^{14}C-activity into the amino acids derived from the soluble leaf proteins following exposure of the leaf to $^{14}CO_2$. This effect of chlorsulfuron was reversed if the stinkweed seedlings were placed with their roots in nutrient solution containing 1 mM of L-valine, L-leucine, and L-isoleucine, 6 to 8 h prior to the herbicide treatment. Under these conditions chlorsulfuron caused an increase in the specific activity of the amino acids derived from the soluble leaf proteins.

On the basis of the results of these experiments, it is difficult to differentiate between two possible effects of chlorsulfuron. In the absence of additional data it could be postulated that chlorsulfuron has an effect on the rate of protein turnover in the leaves. A lower turnover rate would result in a lower specific activity of the proteins. Another hypothesis would be to attribute the observed reduction in the incorporation of ^{14}C-activity into the leaf proteins to the lower specific activity of the soluble amino acids (Table 13), the building blocks of these proteins.

4.9 General discussion

The mode of action of chlorsulfuron involves an inhibition of the biosynthesis of the branched-chain amino acids L-valine and L-isoleucine (Ray, 1984a). The results of the experiments in which the growth of stinkweed seedlings was monitored on a continuous basis, and of the assimilate translocation experiments provide additional evidence for the observation that an effect on this biosynthetic pathway is one of the keys to understanding the mechanism of action of this herbicide. All of the chlorsulfuron-induced events, both in intact seedlings and in excised leaves, could be prevented if the plants were supplied with L-valine, L-leucine, and L-isoleucine.

One of the effects of a 1 µg application of chlorsulfuron to a single leaf of a susceptible plant was a reduction in assimilate transport out of the treated leaf. Figure 27 schematically relates the mechanism of action of chlorsulfuron to some of the physiological and biochemical processes involved in the fixation of CO_2 and the

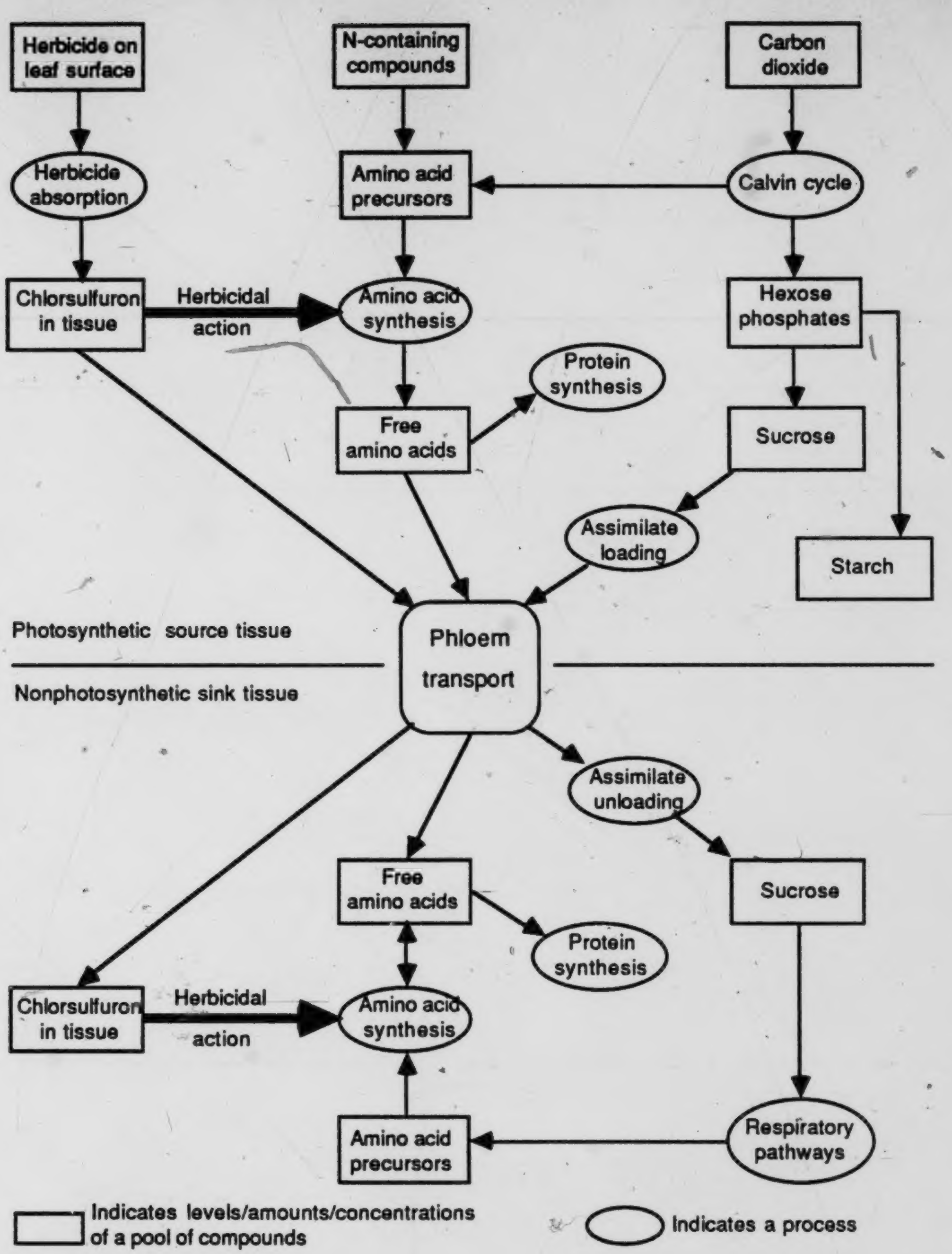


Figure 27. Diagram illustrating the relationship between the mode of action of chlorsulfuron, the fixation of carbon dioxide, and the translocation of assimilate from source to sink tissue.

transport of assimilates from source tissues to sinks. Although the model does not completely reflect the experimental systems used in this study, for the purposes of this discussion it is considered to be adequate.

After application of the herbicide to the leaf surface, herbicide molecules move into the leaf tissue, eventually ending up in the cytoplasm of the individual cells. Depending upon the plant species, the herbicide molecules are metabolized, or remain unchanged (Sweetser *et al.*, 1982). In the case of stinkweed, 90% of the chlorsulfuron molecules remained unchanged after 24 h and about 45% after five days.

Within the cells the chlorsulfuron molecules presumably bind to the enzyme acetolactate synthase (ALS), effectively inhibiting the biosynthesis of L-valine and L-isoleucine. It is postulated that the immediate result of this inhibition is a decrease in the concentration of these amino acids in the tissue. Although no evidence for this was obtained in stinkweed seedlings treated with chlorsulfuron, such a decrease in the concentrations of branched chain amino acids was observed in corn suspension cultures growing in 1 μM imazapyr, a herbicide that also inhibits ALS (Anderson and Hibberd, 1985). The result of the decrease in the levels of branched-chain amino acids is a reduction in the rate of protein biosynthesis. Although the reduction in the incorporation of ^{14}C-activity into the proteins of chlorsulfuron-treated leaf tissue can be interpreted in several ways (see section 4.7), the magnitude of the reduction (43%; Table 15) would suggest that this is not only due to the reduction in the specific activity of the amino acids used for protein synthesis (36%; Table 13). In the light of (a) the relatively short time period after the application of the herbicide (24 h), and (b) the considerably longer turnover rate of leaf proteins (assumed to be in the order of days) (Davies and Humphrey, 1978), the results imply a reduction in the rate of protein biosynthesis.

The details of how the inhibition of the biosynthesis of branched-chain amino acids, and consequently of proteins, is related to the reduction in the transport of assimilates out of the herbicide-treated leaf have not emerged in this study. All that has become abundantly clear is that when the branched chain amino acids were supplied to the treated tissue, the reduction in assimilate transport does not occur.

The increase in the concentration of sugars, in particular of sucrose, in the treated leaves suggests that the effect of chlorsulfuron is not first all an effect on the synthesis of sugars, but more likely on their loading into the phloem. On the basis of the mass flow hypothesis of phloem transport, it can be assumed that once the sucrose has been loaded into the phloem tissue, it should be translocated, unless there is an effect on either the unloading in the sink tissue, or there is an obstruction in the phloem between source and sink (Delrot and Bonnemain, 1985; Giaquinta, 1983). The fact that the chlorsulfuron-induced reduction in assimilate transport also occurs in excised leaves strongly suggests that the herbicide has an effect on the loading of assimilates into the phloem.

Is the effect of chlorsulfuron on the transport of assimilates solely an effect on the source tissue, or is there also an effect on the sink? Assuming that the onset of an effect of the herbicide is related to its concentration in the tissue, the answer to this question must combine the information on the effect on assimilate transport with the information about the concentration of chlorsulfuron in both the source and the sink tissue. Table 16 combines the results of the relevant experiments conducted in this study.

Six hours after the application of chlorsulfuron to intact stinkweed seedlings there was no reduction in assimilate transport, either out of a herbicide-treated leaf or out of a leaf adjacent to the treated leaf (for the purpose of this discussion considered to be the sink). This suggests that either the concentration of chlorsulfuron was too low to cause an effect, or there was a delay before an effect could be demonstrated. Twelve hours after application, chlorsulfuron reduced assimilate transport out of the treated leaf. No effect on the assimilate transport out of the leaf adjacent to the herbicide-treated leaf could be observed, however. At 24 h, the transport of assimilates out of both types of leaves was inhibited. However, if the adjacent leaf was excised 24 h after application, there was no reduction in the exudation of assimilates. Assuming that at the time of the exposure to $^{14}CO_2$ the same amount of chlorsulfuron was present in each leaf, an argument could be made that the removal of the sink, by excising the leaf, caused a decrease in the chlorsulfuron-induced reduction in assimilate transport. Apparently, the concentration of chlorsulfuron in the tissue of the excised adjacent leaf was too low to cause an effect

Table 16. Comparison between the effect of chlorsulfuron on assimilate translocation in intact stinkweed seedlings, on the exudation by excised leaves, and on the concentration of chlorsulfuron in the assimilate source tissue.

Experiment	Time between chlorsulfuron application and exposure to $^{14}CO_2$	Relationship between leaf treated with chlorsulfuron and leaf exposed to $^{14}CO_2$	Concentration of chlorsulfuron in leaf exposed to $^{14}CO_2$[a]		Effect on assimilate export[b]
	(h)		(pmole/mg)		
Intact seedlings[c]	6	same	1.19	(0.33)	-
		adjacent	0.09	(0.02)	-
	12	same	1.39	(0.21)	I
		adjacent	0.21	(0.09)	-
	24	same	4.21	(0.77)	I
		adjacent	0.24	(0.08)	I
Excised leaves[d]	24	same	4.21	(0.77)	I
		adjacent	0.24	(0.08)	-

[a] Based on data presented in Table 5 (Means with standard errors in parentheses).
[b] A hyphen indicates an absence of an effect; I indicates a reduction in assimilate export.
[c] Based on data presented in Figure 18.
[d] Based on data presented in Figure 23.

on assimilate exudation. Hence, these results would suggest that an effect of chlorsulfuron on the sink tissue cannot be completely eliminated in an attempt to explain the reduction in assimilate transport out of the treated leaves of intact seedlings.

One of the consequences of the chlorsulfuron-induced reduction in the rate of assimilate transport out of the treated leaves is a reduction in the rate at which the herbicide molecules themselves are translocated out of the treated tissue. Devine *et al.* (1987) have suggested that on the basis of its physico-chemical properties chlorsulfuron should be translocated more readily than it is in whole plants. Assuming that chlorsulfuron has no direct effect, i.e., an effect not mediated through the inhibition of branched-chain amino acid biosynthesis, on the loading of the herbicide molecules into the phloem tissue, supplying the treated plants with branched-chain amino acids should increase the rate of chlorsulfuron transport.

This self-limiting aspect of chlorsulfuron transport as a consequence of its phytotoxic action might explain the difference in the translocation of chlorsulfuron between the tolerant eastern black nightshade and the susceptible velvetleaf as reported by Hageman and Behrens (1984a). At 24 and 48 h after application, chlorsulfuron translocation was about 20% greater in eastern black nightshade than in velvetleaf. Presumably, the rate of assimilate translocation out of the treated leaves of velvetleaf was reduced by the herbicide, resulting in a lower rate of translocation of the herbicide.

5. SUMMARY AND CONCLUSIONS

Stinkweed is susceptible to the herbicide chlorsulfuron. The susceptibility of stinkweed can be attributed to the slow rate of metabolism of chlorsulfuron to nonphytotoxic products. At 24 h after application approximately 88% of the applied chlorsulfuron was still in the phytotoxic form; at 120 h after application this had decreased to 45%. This is in contrast to the rate of chlorsulfuron metabolism in wheat, a species resistant to chlorsulfuron, in which at 120 h after application only 6% was still in the phytotoxic form.

One of the visible effects of chlorsulfuron on stinkweed seedlings was a rapid reduction in shoot growth, resulting in stunted plants. A spot application of 7 ng (20 picomoles) per seedling to the foliage was sufficient to result in a 50% reduction in plant height 14 days later.

Chlorsulfuron was absorbed slowly by the leaves of stinkweed seedlings. Twenty four hours after application, 12 - 25% of the applied amount of herbicide had been absorbed. Translocation out of the treated leaf was limited. Only 1.7 - 2.8% of the applied amount had moved out of the treated leaf 24 h after application. The distribution of the translocated chlorsulfuron was as follows (in terms of the total amount applied): 0.15% was recovered in the shoot apex, 0.2% in the roots, 0.75% in the leaf adjacent to the herbicide-treated leaf, and 1.7% in the remainder of the shoot. Absorption via the roots, and subsequent translocation to the shoot occurred much more readily.

In plants exhibiting stunted growth the chlorsulfuron concentration in the apical tissue was 1 to 5 picomoles per mg of tissue fresh weight. A specific consequence of chlorsulfuron treatment was a reduction in ^{14}C incorporation into amino acids in the apical tissue when the foliage was exposed to $^{14}CO_2$. It is postulated that this reduction in ^{14}C incorporation into the amino acids is indicative of an effect of the herbicide on amino acid biosynthesis. This in turn results in an inhibition of the synthesis of proteins. The inhibition of this latter process manifests itself as a reduction in the formation of new tissue, i.e., an inhibition of growth.

Chlorsulfuron treatment of a single leaf reduced the transport of assimilates out of that leaf. Twelve hours after a spot application of 1 µg, assimilate translocation was reduced by 30%. This reduction in assimilate transport also occurred in excised chlorsulfuron-treated leaves.

In chlorsulfuron-treated excised leaves, the concentration of sucrose had increased from 1.7 to 4.2 nmoles/mg, 30 h after application of the herbicide. Concomitantly, the concentration of free amino acids had increased from 16.8 to 28.6 nmoles/mg. Incorporation of ^{14}C-activity into these amino acids, and in the amino acids derived from soluble proteins extracted from the treated leaves, was reduced. The increase in the concentration of sucrose in the chlorsulfuron-treated leaves, combined with the absence of an effect of chlorsulfuron on carbon dioxide fixation, suggests that the observed reduction in assimilate transport is not due to an effect on the synthesis of assimilates, but rather on their movement out of the leaves.

Although the possibility that the effect of chlorsulfuron on assimilate transport is due to an effect on the assimilate sink tissue has not been eliminated, there appears to be a definite effect of the herbicide on the assimilate source tissue, presumably on the loading of assimilates into the phloem.

The effect of chlorsulfuron on the export of assimilates out of a herbicide-treated leaf provides an explanation for the limited phloem mobility of the herbicide in susceptible species.

Supplying branched-chain amino acids to stinkweed seedlings prior to the application of chlorsulfuron prevented the occurrence of all effects described. This observation is consistent with the evidence that most chlorsulfuron-induced effects appear to occur as a direct result of an inhibition of the synthesis of the branced-chain amino acids (Ray, 1984a).

Although no direct link has been established between the inhibition of the biosynthesis of branched-chain amino acids and the reduction in the rate of assimilate transport out of a treated leaf, this effect might be mediated through an inhibition of protein synthesis, caused by a lack of availability of a full complement of required amino acids in the chlorsulfuron-treated tissue.

BIBLIOGRAPHY

Amrhein, N. B. Deus, P. Gehrke, and H.C. Steinrücken. 1980. The site of the inhibition of the shikimate pathway by glyphosate. II. Interference by glyphosate with chorismate formation *in vivo* and *in vitro*. Plant Physiol. 66:830-834.

Amrhein, N., H. Holländer-Czytko, J. Leifeld, A. Schulz, H.C. Steinrücken, and H. Topp. 1982. Inhibition of the shikimate pathway by glyphosate. *In* A.M. Boudet, R. Ranjeva, eds. Journées Internationales d'Études et Assemblées Générals, Bulletin de Liaison, Vol 11. Groupe Polyphenols, Toulouse, France. pp. 21-30.

Anderson, P.C. and K.A. Hibberd. 1985. Evidence for the interaction of imidazolinone herbicide with leucine, valine, and isoleucine metabolism. Weed Sci. 33:479-483.

Anderson, P.C. and K.A. Hibberd. 1985. Herbicide resistance in plants. Eur. Pat. Applic. 154204, 70 pp.

Anon. 1979. Herbicide Handbook of the WSSA. Weed Science Society of America. Champaign, Illinois. 479 pp.

Anon. 1980. Product Information Bulletin. E.I. DuPont de Nemours & Co. Inc. Wilmington, Delaware.

Ashton, F.M., O.T. de Villiers, R.K. Glenn, and W.B. Duke. 1977. Localization of metabolic sites of action of herbicides. Pestic. Biochem. Physiol. 7:122-141.

Atkins, C.A. and D.T. Canvin. 1971. Photosynthesis and CO_2 evolution by leaf discs: gas exchange, and ion exchange fractionation of ^{14}C-labeled photosynthetic products. Can. J. Bot. 49:1225-1234.

Bayer, E., K.H. Gugel, K. Hägele, H. Hagemaier, S. Jessipow, W.A. König, and H. Zähner. 1972. Stoffweckselprodukte von Mikroorganismen. Phosphinothricin und Phosphinothricyl-alanyl-alanin. Helv. Chim. Acta 55:224-239.

Berlin, J. and L. Witte. 1981. Effects of glyphosate on shikimic acid accumulation in tobacco cell cultures with low and high yields of cinnamoyl putrescines. Z. Naturf. 36C:210-214.

Bestman, H.D., M.D. Devine, and W.H. Vanden Born. 1987. Extraction and separation of chlorsulfuron and its metabolites from treated plants. Weed Sci. 35:22-26.

Blair, A.M. and T.D. Martin. 1988. A review of the activity, fate and mode of action of sulfonylurea herbicides. Pestic. Sci. 22:195-219.

Boocock, M.R. and J.R. Coggins. 1983. Kinetics of 5-enolpyruvyl-shikimate 3-phosphate synthase inhibition by glyphosate. FEBS Letters 154:127-133.

Chaleff, R.S. and T.B. Ray. 1984. Herbicide-resistant mutants from tobacco cell cultures. Science 223:1148-1151.

Chaleff, R.S. and C.J. Mauvais. 1984. Acetolactate synthase is the site of acton of the two sulfonylurea herbicides in higher plants. Science 224:1443-1445.

Chua, N-H. 1973. Electrophoretic analysis of chloroplast proteins. Methods Enzymol. 69:434-446.

Cole, D.J., A.D. Dodge, and J.C. Caseley. 1980. Some biochemical effects of glyphosate in plant meristems. J. Exp. Bot. 26:138-144.

Cole, D.J. 1985. Mode of acton of glyphosate - a literature analysis. *In* E. Grossbard and D. Atkinson, eds. The Herbicide Glyphosate. Butterworths, London. pp. 48-74.

Comai, L. D. Facciotti, W.R. Hiatt, G. Thompson, R.E. Rou, and D.M. Stalker. 1985. Expression in plants of a mutant *aroA* gene from *Salmonella typhimurium* confers tolerance to glyphosate. Nature 317:741-744.

Davies, D.A. and T.J. Humphrey. 1978 Amino acid recycling in relation to protein turnover. Plant Physiol. 61:54-58.

De Felice, M., C.T. Lago, D.H. Squires, and J.M Calvo. 1982. Acetohydroxyacid synthase isoenzymes of *Escherichia coli* K-12 and *Salmonella typhimurium*. Ann. Microbiol. 133A:251-256.

Delrot, S. and J-L. Bonnemain. 1985. Mechanism and control of phloem transport. Physiol. Vég. 23:199-220.

de Villiers, O.T., M.L. VandenPlas, and H.M. Koch. 1980. The effect of DPX-4189 on biochemical processes in isolated leaf cells and chloroplasts. Proc. Brit. Crop Protection Conf. - Weeds. pp. 237-242.

Devine, M.D., H. D. Bestman, J.C. Hall, and W.H. Vanden Born. 1984. Leaf wash techniques for estimation of foliar absorption of herbicides. Weed Sci. 32:418-425.

Devine, M.D. and W.H. Vanden Born. 1985. Absorption, translocation, and foliar activity of clopyralid and chlorsulfuron in Canada thistle (*Cirsium arvense*) and perennial sowthistle (*Sonchus arvensis*). Weed Sci. 33:524-530.

Devine, M.D., H.D. Bestman, and W.H. Vanden Born. 1987. Uptake and accumulation of the herbicides chlorsulfuron and clopyralid in excised pea root tissue. Plant Physiol. 85:82-86.

Dickson, R.E. 1979. Analytical procedures for the sequential extraction of ^{14}C-labeled constituents from leaves, bark and wood of cottonwood plants. Physiol. Plant. 45:480-488.

Donald, W.W. 1984. Chlorsulfuron effects on shoot growth and root buds of Canda thistle (*Cirsium arvense*). Weed Sci. 32:42-50.

Duke, S.O. and R.E. Hoagland. 1978. Effects of glyphosate on metabolism of phenolic compounds I. Induction of phenylalanine ammonia-lyase activity in dark-grown maize roots. Plant Sci. Letters 11:185-190.

Ekanayake, A. , R.L. Wickremasinghe, and H.D.S. Liyanoge. 1979. Studies on the mechanism of herbicidal action of *N*-(phosphono-methyl)glycine. Weed Res. 19:39-43.

Falco, S.C. and K.S. Dumas. 1985. Genetic analysis of mutants of *Saccharomyces cerevisiae* resistant to the herbicide sulfometuron methyl. Genetics 109:21-35.

Fedtke, C. 1982. Biochemistry and Physiology of Herbicide Action. Springer-Verlag, New York. 191 p.

Fedtke, C. and A. Trebst. 1986. Advances in understanding herbicide mode of action. IUPAC, 1986.

Fellows, R.J. and J.A.D. Zeevaart. 1983. Comparison of ethylenediaminetetra-acetate-enhanced exudation from detached and translocation from attached bean leaves. Plant Physiol. 71:716-718.

Giaquinta, R.T. 1983. Phloem loading of sucrose. Ann. Rev. Plant Physiol. 34:347-387.

Gresshoff, P.M. 1979. Growth inhibition by glyphosate and reversal of its action by phenylalanine and tyrosine. Austr. J. Plant Physiol. 6:177-185.

Groussol, J., S. Delrot, P. Caruhel, and J-L. Bonnemain. 1986. Design of an improved exudation method for phloem sap collection and its use for the study of phloem mobility of pesticides. Physiol. Vég. 24:123-133.

Haderlie, L.C., J.M. Widholm, and F.W. Slife. 1977. Effect of glyphosate on carrot and tobacco cells. Plant Physiol. 60:40-49.

Hageman, L.H. and R. Behrens. 1981. Response of small-grain cultivars to chlorsulfuron. Weed Sci. 29:414-420.

Hageman, L.H. and R. Behrens. 1984a. Basis for response differences of two broadleaf weeds to chlorsulfuron. Weed Sci. 32:162-167.

Hageman, L.H. and R. Behrens. 1984b. Chlorsulfuron induction of leaf abscission in velvetleaf (*Abutilon theophrasti*). Weed Sci 32:132-137.

Hatzios, K.K. and C.M. Howe. 1982. Influence of the herbicides hexazinone and chlorsulfuron on the metabolism of isolated soybean leaf cells. Pestic. Biochem. Physiol. 17:207-214.

Haughn, G.W. and C. Somerville. 1986. Sulfonylurea-resistant mutants of *Arabidopsis thaliana*. Mol. Gen. Genet. 204:430-434.

Hauptman, R.M., G. della-Cioppa, A.G. Smith, G.M. Kishore, and J.M. Widholm. 1988. Expression of glyphosate resistance in carrot somatic hybrid cells through the transfer of an amplified 5-enol-pyruvylshikimic acid-3-phosphate synthase gen. Mol. Gen. Genet. 211:357-363.

Haughn, G.W., J. Smith, B. Mazur, and C. Somerville. 1988. Transformation with a mutant *Arabidopsis* acetolactate synthase gene renders tobacco resistant to sulfonylurea herbicides. Mol. Gen. Genet. 211:266-271.

Hay, J.R. 1980. Pesticides and herbicides - Present and anticipated use by 1990. A paper prepared for Canada West Foundation, Calgary.

Herrmann, M. D. Kotzias, and F. Korte. 1985. Photochemical behaviour of chlorsulfuron in water and in adsorbed phase. Chemosphere 14:3-8.

Hilton, J.L., P.C. Kearney, and B.N. Ames. 1965. Mode of action of the herbicide 3-amino-1,2,4-triazole (Amitrole): inhibition of an enzyme of histidine biosynthesis. Archiv. Biochem. Biophys. 112:544-547.

Hilton, J.L. 1969. Inhibitions of growth and metabolism by 3-amino-1,2,4-triazole (Amitrole). J. Agric. Food Chem. 17:182-198.

Hoagland, D.R. and D.I. Arnon. 1950. The water culture method for growing plants without soil. Calif. Expt. Sta. Circ. 347. 32 pp.

Hoagland, R.E., S.O. Duke, and D. Elmore. 1978. Effect of glyphosate on metabolism of phenolic compounds. II. Influence on soluble hydroxyphenolic compound. free amino acid, and soluble protein levels in dark-grown maize roots. Plant Sci. Letters 13:291-299.

Hoagland, R.E., S.O. Duke, and D. Elmore. 1979. Effect of glyphosate on metabolism of phenolic compounds. III. Phenylalanine ammonia-lyase activity, free amino acids, soluble protein and hydroxyphenolic compounds in axes of dark-grown soybeans. Physiol. Plant. 46:357-366.

Holländer, H. and N. Amrhein. 1980. The site of inhibition of the shikimate pathway by glyphosate. I. Inhibition by glyphosate of phenylpropanoid synthesis in buckwheat (*Fagopyrum esculentum* Moench.). Plant Physiol. 66:823-829.

Hutchinson, J.M., R. Shapiro, and P.B. Sweetser. 1984. Metobolism of chlorsulfuron by tolerant broadleaves. Pestic. Biochem. Physiol. 22:243-247.

Jaworski, E.G. 1972. Mode of action of *N*-(phosphonometyl)glycine: inhibition of aromatic amino acid biosynthesis. J. Agric. Food Chem. 20:1195-1198.

King, R.W. and J.A.D. Zeevaart. 1974. Enhancement of phloem exudation from cut petioles by chelating agents. Plant Physiol. 53:96-103.

LaRossa, R.A. and J.V. Schloss. 1984. The sulfonylurea herbicide sulfometuron methyl is an extremely potent and selective inhibitor of acetolactate synthase in *Salmonella typhimurium*. J. Biol. Chem. 259:8753-8757.

LaRossa, R.A. and S.C. Falco. 1984. Amino acid biosynthetic enzymes as targets of herbicide action. Trends Biotechnol. 2:158-161.

LaRossa, R.A. and D.R. Smulski. 1984. *ilvB*-Encoded acetolactate synthase is resistant to the herbicide sulfometuron methyl. J. Bacteriol. 169:391-394.

Leason, M., D. Cunliffe, D. Parkin, P.J. Lea, and B.J. Miflin. 1982. Inhibition of pea leaf glutamine synthetase by methionine sulphoximine, phosphinothricin, and other glutamate analogues. Phytochem. 21:855-857.

Levitt, G., H.L. Ploeg, R.C. Weigel, Jr., and D.J. Fitzgerald. 1981. 2-chloro-*N*-[(4-methoxy-6-methyl-1,3,5-triazin-2-yl)aminocarbo-nyl]benzenesulfonamide, a new herbicide. J. Agric. Food Chem. 29:416-418.

Levitt, G. 1982. Sulfonylureas: new high potency herbicides. *In* Pesticide Chemistry: Human Welfare and the Environment Vol. 1 pg 243-250. Vol 1, Synthesis and structure-activity relationships. Volume editors: P. Doyle and T. Fujita. Proceedings of the 5th International Congress of Pesticide Chemistry. Kyoto, Japan 29 August-4 Sept. 1982. Editors in Chief: J. Miyanoto and P.C. Kearney. Pergamon Press, New York.

Lowry, O.H., N.J. Rosebrough, A.L. Farr, and R.J. Randall. 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. 193:265-275.

McWhorter, C.G. and J.L. Hilton. 1967. Alterations in amino acid content caused by 3-amino-1,2,4-triazole. Physiol. Plant. 20:30.

Moore, S. 1968. Amino acid analysis: aqueous dimethyl sulfoxide as solvent for the ninhydrin reaction. J. Biol. Chem. 243:6281-6283.

Mousdale, D.M. and J.R. Coggins. 1984. Purification and properties of 5-enolpyruvylshikimate 3-phosphate synthase from seedlings of *Pisum sativum* L. Planta 160:78-83.

Muhitch, M.J., D.L. Shaner, and M.A. Stidham. 1987. Imidazolinones and acetohydroxyacid synthase from higher plants. Properties of the enzyme from maize suspension culture cells and evidence for the binding of imazapyr to acetohydroxyacid synthase *in vivo*. Plant Physiol. 83:451-456.

Neville, D.M., Jr. 1971. Molecular weight determination of a protein-dodecyl sulfate complex by gel electrophoresis in a discontinuous buffer system. J. Biol. Chem. 246-6328-6334.

Nilsson, G. 1977. Effect of glyphosate on the amino acid content in spring wheat plants. Swed. J. Agric. Res. 7:153-157.

OSullivan, P.A. 1982. Response of various broad-leaved weeds, and tolerance of cereals, to soil and foliar applications of DPX-4189. Can. J. Plant Sci. 62:715-724.

Ray, T.B. 1980. Studies on the mode of action of DPX-4189. Proc. Brit. Crop Protection Conf. - Weeds. pp. 7-14.

Ray, T.B. 1982a. The mode of action of chlorsulfuron: the lack of direct inhibition of plant DNA synthesis. Pestic. Biochem. Physiol. 18:262-266.

Ray, T.B. 1982b. The mode of action of chlorsulfuron: a new herbicide for cereals. Pestic. Biochem. Physiol. 17:10-17.

Ray, T.B. 1984a. Site of action of chlorsulfuron. Inhibition of valine and isoleucine biosynthesis in plants. Plant Physiol. 75:827-831.

Ray, T.B. 1984b. The primary site of action of the herbicide chlorsulfuron. Abstract 223, Weed Science Society of America.

Ribo, J.M. 1988. The octanol/water partition coefficient of the herbicide chlorsulfuron as a function of pH. Chemosphere 17:709-715.

Roisch, U. and F. Lingens. 1974 Effect of the herbicide *N*-(phosphonomethyl)-glycine on the biosynthesis of aromatic amino acids. Angew. Chemie Intern. Ed. 13:400.

Roisch, U. and F. Lingens. 1980. The mechanism of action of the herbicide *N*-(phosphonomethyl)glycine: its effect on the growth and the enzymes of aromatic amino acid biosynthesis in *Escherichia coli*. Hoppe-Seyler's Zeitschrift für Physiologische Chemie 361:1049-1058.

Rost, T.L. 1984. The comparative cell cycle and metabolic effect of chemical treatments on root tip meristems. III. Chlorsulfuron. J. Plant Growth Regul. 3:51-63.

Rost, T.L. and T. Reynolds. 1985. Reversal of chlorsulfuron-induced inhibition of mitotic entry by isoleucine and valine. Plant Physiol. 77:481-482.

Rubin, J.L., C.G. Gaines, and R.A. Jensen. 1982. Enzymological basis for herbicidal action of glyphosate. Plant Physiol. 70:833-839.

Rubin, J.L., C.G. Gaines, and R.A. Jensen. 1984. Glyphosate inhibition of 5-enolpyruvylshikimate 3-phosphate synthase from suspension-cultured cells of *Nicotiana silvestris*. Plant Physiol. 75:839-845.

Saxena, P.K. and J. King. 1988. Herbicide resistance in *Datura innoxia*: cross resistance of sulfonylurea-resistant cell lines to imidazolinones. Plant Physiol. 86:863-867.

Scheel, D. and J.E. Casida. 1985. Sulfonyl urea herbicides: growth inhibition in soybean cell suspension cultures and in bacteria correlated with block in biosynthesis of valine, leucine, or isoleucine. Pestic. Biochem. Physiol. 23:398-412.

Shaner, D.L. and J.L. Lyon. 1980. Interaction of glyphosate with aromatic amino acids on transpiration in *Phaseolus vulgaris*. Weed Sci. 28:31-35.

Shaner, D.L. P.C. Anderson, and M.A. Stidham. 1984. Imidazolinones. Potent inhibitors of acetohydroxyacid synthase. Plant Physiol. 76:545-546.

Shaner, D.L. and M.L. Reider. 1986. Physiological responses of corn (*Zea mays*) to AC-243,997 in combination with valine, leucine, and isoleucine. Pestic. Biochem. Physiol. 25:248-257.

Shah, D.M, R.B. Horsch, H.J. Klee, G.M. Kishore, J.A. Winter, N.E. Tumer, C.M. Hironaka, P.R. Sanders, C.S. Gasser, S.Aykent, N.R. Siegel, S.G. Rogers, and R.T. Fraley. 1986. Engineering herbicide tolerance in transgenic plants. Science 233:478-481.

Simpson, R.J., M.R. Neuberger, and T-Y. Liu. 1976. Complete amino acid analysis of protein from a single hydrolysate. J. Biol. Chem. 251:1936-1940.

Skinner, M.K. and M.D. Griswold. 1983. Fluorographic detection of radioactivity in polyacrylamide gels with 2,5-diphenyloxazole in acetic acid and its comparison with existing procedures. Biochem. J. 209:281-284.

Slates, R.V. 1983. Determination of chlorsulfuron residues in grain, straw, and green plants of cereals by high performance liquid chromatography. J. Agric. Food Chem. 31:113-117.

Smart, C.C. and N. Amrhein. 1987. Ultrastructural localisation by protein A-gold immunocytochemistry of 5-enolpyruvylshikimic acid 3-phosphate synthase in a plant cell of a culture which overproduces the enzyme. Planta 170:1-6.

Spiro, R.G. 1966. Analysis of sugars found in glycoproteins. Methods Enzymol. 8:3-26.

Streibig, J.C. 1981. A method for determining the biological effect of herbicide mixtures. Weed Sci. 29:469-473.

Suttle, J.C. and D.R. Schreiner. 1981. Effects of DPX-4189 (2-chloro-N-((4-methoxy-6-methyl-1,3,5-triazin-2-yl)amino-carbonyl)benzene-sulfonamide) on anthocyanin synthesis, phenylalanine ammonia lyase activity, and ethylene production in soybean hypocotyls. Can. J. Bot. 60:741-745.

Suttle, J.C. 1983. Effect of chlorsulfuron on ethylene evolution from sunflower seedlings. J. Plant Growth Regul. 2:31-45.

Suttle, J.C., H.R. Swanson, and D.R. Schreiner. 1983. Effect of chlorsulfuron on phenylpropanoid metabolism in sunflower seedlings. J. Plant Growth Regul. 2:137-149.

Sweetser, P.B., G.S. Schow, and J.M. Hutchinson. 1982. Metabolism of chlorsulfuron by plants: biological basis for selectivity of a new herbicide for cereals. Pestic. Biochem. Physiol. 17:18-23.

Wallevik, K., J.C. Jensenius, I. Anderson, and A.M. Poulsen. 1982. A simple and reliable method for the drying of polyacrylamide slab gels. J. Biochem. Biophys. Methods 6:17-21.

Zahnow, E.W. 1982. Analysis of the herbicide chlorsulfuron in soil by liquid chromatography. J. Agric. Food Chem. 30:854-857.

APPENDIX

Equation used to describe the relationship between the chlorsulfuron dose and the height, fresh and dry weight of stinkweed seedlings, 14 days after treatment.

$$Y = \frac{UASYM - LASYM}{\left[1 + e^{(-2(A + B \cdot LNDOSE))}\right]} + LASYM$$

Y = dependent variable (height, fresh weight, dry weight)
UASYM = upper asymptote
LASYM = lower asymptote
A = constant
B = constant
LNDOSE = logarithm of the chlorsulfuron dose

Y	UASYM[a]		LASYM[a]		A[a]		B[a]		Loss function[b]
Height	98.4	(4.1)	11.4	(2.2)	3.18	(1.00)	-1.68	(0.51)	12,941
Fresh Weight	99.8	(7.8)	12.4	(9.5)	2.17	(0.72)	-0.478	(0.16)	56,371
Dry Weight	99.8	(7.2)	12.4	(11.5)	2.17	(0.64)	-0.478	(0.16)	56,371

[a] Means with standard errors in parentheses.

[b] This value indates the final value of the least squares loss function.

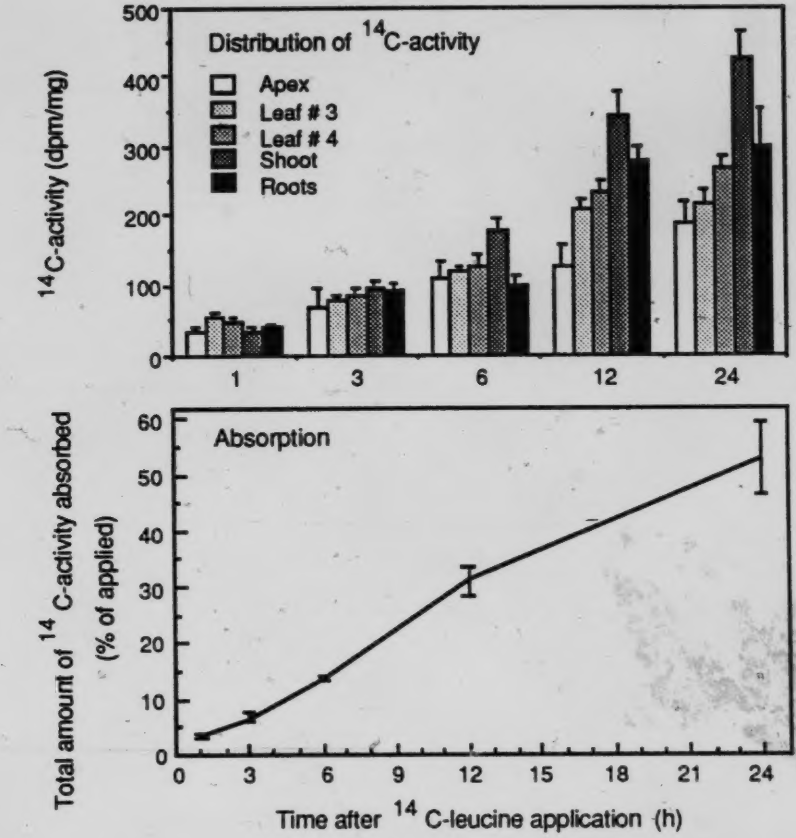


Figure A1. Absorption and distribution of ^{14}C-activity following application of ^{14}C-leucine to the roots of stinkweed seedlings growing in nutrient solution containing 1 mM of each of the amino acids L-valine, L-leucine, and L-isoleucine. The data, means and standard errors, are the results of one run with three seedlings per treatment. 1000 Dpm of ^{14}C-activity corresponds to approximately 6 nmoles of L-leucine.